

AN INTRODUCTION TO BIOCHEMISTRY

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TO
SIR FREDERICK GOWLAND HOPKINS, o.m.

*What is but now compleat and done
Was long before resolved on.*

THE CHYMICAL MARRIAGE OF CHRISTIAN ROSENCREUTZ

" We carry with us the wonders we seek
without us : there is all Africa and her
prodigies in us."

THOMAS BROWNE

PREFACE TO THE THIRD EDITION

FOR this edition, all the chapters have been revised and many sections have been added. More emphasis has been placed on certain subjects of special interest in clinical medicine, namely: acid-base balance, animal calorimetry, carbon dioxide transport, energy transformation, blood chemistry, bone-formation, food absorption, detoxication. A new chapter on "Tissue Chemistry" has been included, and the chapter on "Nutrients" is almost entirely rewritten. New and original reactions described are the methylamine test for lactose and maltose, the dinitrobenzene test for dienol compounds, and the chloroimide test for uric acid.

The demand for references has been met, in part, by the provision of lists of reviews and monographs; references by name and date throughout the text are too numerous to be specified with full details, but can be traced in standard sources of information such as: *British Chemical and Physiological Abstracts*; *Abstracts of the American Chemical Society*; *Nutrition Abstracts and Reviews*; *Biological Abstracts*; *Annual Review of Biochemistry*; *Annual Review of Physiology*.

Descriptions of practical methods suitable for clinical application will be found in *Laboratory Directions in Biochemistry*, by V. C. Myers.

For advice, information and literature I am indebted to many correspondents, including Professors V. Arreguine, A. C. Chibnall, E. J. Conway, E. C. Dodds, D. Harrison, A. Hunter, Victor C. Myers, Sir Joseph Barcroft, Mr. A. L. Bacharach, Mr. Julian Huxley, Sir Jack Drummond, Doctors M. Dixon, L. J. Harris, J. Needham, J. H. Quastel, J. Foley, Karl P. Link, R. A. McCance, Martin H. Fischer, and E. Forbes.

Figs. 1 and 2 are reproduced, by kind permission, from Professor Niels Bjerrum's *Inorganic Chemistry* (translated by R. P. Bell), and Fig. 3 is from Dr. Sherwood Taylor's *Organic Chemistry*.

Finally, I am glad to have the opportunity of recording how grateful I am to my publishers for the trouble they have taken and the help they have given.

TRINITY COLLEGE
DUBLIN

W. R. F.

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AN INTRODUCTION TO BIOCHEMISTRY

PART I

ELEMENTS AND INORGANIC COMPOUNDS

CHAPTER 1

THE SUBJECT-MATTER OF BIOCHEMISTRY

"Definitions, formulæ (some would add, creeds) have their use in any society in that they restrain the ordinary unintellectual man from making himself a public nuisance with his private opinions."

ARTHUR QUILLER-COUCH.

Biochemistry is the study and the interpretation of the chemical changes associated with life. Life is indefinable, but is recognisable by its manifestation in material organisms composed of two or three dozen relatively common chemical elements. These organisms are autonomous systems displaying the following properties: adaptiveness, growth, repair, reproductiveness. From the biochemical standpoint, *an organism is a system that maintains its identity by means of self-controlled physical and chemical changes.*

Physics and Chemistry.—Matter may undergo three kinds of transformation: (1) molecular rearrangement, as in the conversion of water into ice; (2) molecular decomposition and atomic rearrangement, as in the electrolysis of water into hydrogen and oxygen; (3) nuclear decomposition, as in the transformation of lithium into helium. A physical change merely involves molecular rearrangement; a chemical change involves atomic rearrangement with the production of new molecules; a sub-atomic change involves rearrangement in electron systems, and production of new atoms. Biochemistry is concerned primarily with the composition of plant and animal structures and products, and the atomic rearrangements taking place in plants and animals. Many of these chemical changes are accompanied by important physical events which are often included in the study of biochemistry, although, strictly they belong to the related sciences of biophysics and physiology.

Development of Biochemistry.—A distinction, between the chemistry of living and non-living substances is implicit in the

writings of the alchemists, one of whom, as Dr. Needham has pointed out, gave biochemistry its charter when he wrote :—

“The Body is a conglomeration of chemical matters ; when these are deranged, illness results, and nought but chemical medicines may cure the same.”

THEOPHRASTUS PARACELSUS, 1527.

In 1675, Nicholas Léméry, the author of the first rational text-book on chemistry, classified the science as *animal*, *vegetable*, and *mineral*. Lavoisier, about 1770, observed that animal and vegetable material differed from minerals in being very rich in carbon, hydrogen, and oxygen. Subsequently, he detected nitrogen and phosphorus in animal matter, and concluded that in this respect it differed from vegetable matter. Later work showed that this conclusion was false ; all plants contain nitrogen and phosphorus, although these elements may be absent from some vegetable products. As a result, animal and plant chemistry were grouped together under the name of *organic chemistry*, in distinction from *inorganic* or *mineral chemistry*.

Early in the nineteenth century it was assumed that a fundamental difference existed between these two classes, organic compounds being the exclusive products of vital activity. Then, in 1828, Wöhler, a young German chemist, accidentally obtained urea from ammonium cyanate, by the action of heat. Urea, the chief nitrogenous solute of mammalian urine, is a typical organic compound ; indeed, from the point of view of a vegetable it would be regarded as the chief useful product of the animal kingdom. Wöhler's startling discovery altered the outlook of chemistry. The synthesis of other vital products gradually followed, and by the end of the century the term *organic chemistry* had lost its original vitalistic implications, and was applied to all the combustible compounds of carbon, irrespective of their natural or artificial origin. In the meantime, the chemistry of animal and plant materials had been pursued under such titles as : medical chemistry, agricultural chemistry, pathological chemistry, and chemical physiology, until the term *biological chemistry* (abbreviated to the hybrid, *biochemistry*) was introduced to include all applications of chemistry to the study of life and its products.

Vitalism, Mechanism, and Organicism.—To a vitalist, the changes in a living organism are not completely explicable in terms of physics and chemistry. To the mechanist, life is a manifestation of higher material properties, which, given suitable conditions, appear along with the other products of chemical reactions, and are equally devoid of purpose. To the organicist, or neo-vitalist, there is neither life-substance nor life-force, but, instead, there is a specific life-

structure within which various changes take place according to the laws of physics and chemistry. These metaphysical considerations are outside the scope of most workers, who well may agree with Whitehead that :—

“The mode of approach to the problem, so far as science is concerned, is merely to ask if molecules exhibit in living bodies properties which are not to be observed amid inorganic surroundings.”

To a disembodied and detached observer, a living organism is only a form in which carbon is collected, stored and oxidised. Why this should occur under these restricted conditions, or whether the organism has any cosmic function other than to delay the dissipation of solar energy, is a problem beyond the horizon of the biochemist, whose task is to find what changes occur and how they are brought about. The existence of life is accepted as a self-evident fact in the hope that man's experience of it will eventually enable him to understand its meaning.

The Four-Dimensional Organism.—In the early period of organic chemistry it was possible to represent compounds by two-dimensional formulæ on paper, but the discovery of optical isomerism made it necessary to employ three-dimensional perspective formulæ to show the distinction between related compounds. The living organism is more complex than a tri-dimensional solid ; it is a structure undergoing changes in time as well as in space, and its composition and pattern at a given moment must be considered in relation to its previous and future history. This concept was present in the mind of Bergson when he wrote : “Doubtless we think with only a small part of our past, but it is with our entire past that we desire, will and act.” Knowledge of the earlier states of an organism enables an observer to predict the immediate future states, and for this reason serial observations are becoming more widely used. When possible, biochemical data, such as gastric acidity or blood sugar values, are presented in the form of time-concentration curves instead of single analyses.

World-Volume.—Regarded as a four-dimensional system, man is an organism occupying a certain amount of space—which, during most of his life, is about 70 litres—for a certain number of years—usually 65–70. By multiplying these values a new quantity, the *world-volume*, is obtained which expresses the entire history of the organism. The world-volume of the human subject is about 5,000 litre-years, starting from the fertilised ovum of 0.004 cu.mm. The composition of an organism at a given instant represents a cross-section of its world-volume at that instant.

Forms of Life.—The *biosphere*, or inhabited surface of the world, is colonised by representatives of two great kingdoms : the animals

and the plants; and also by the vast republic of micro-organisms, most of which have their home and factory in the soil. All forms of life investigated are assembled chiefly from water, proteins, carbohydrates and lipides; and all are engaged in a continuous chemical traffic with their environment.

In monocellular organisms the affairs of life are carried out by single microscopic units; higher organisms are characterised by progressive differentiation of structures and functions, as shown by the presence of specialised tissues, organs and systems, designed to serve the general purpose of life, which is survival.

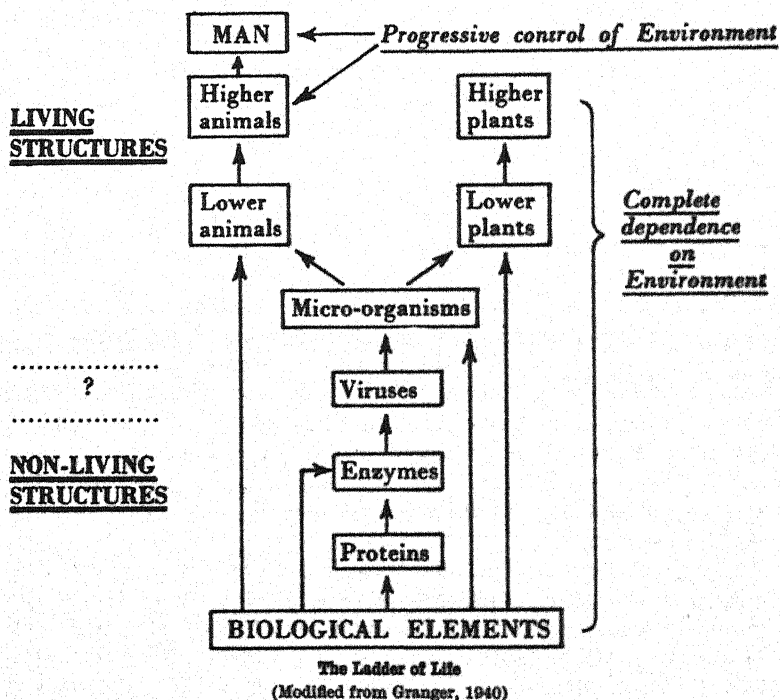
While both animals and plants are constructed of identifiable compounds undergoing transformations in measurable and predictable ways, the highest animals display increasing capacity for selecting and modifying their environments. Bacteria are monocellular organisms of microscopic dimensions, the usual order being 1-2 microns. The cell membrane is generally strong, and enables some varieties to withstand extremes of temperature from 0° C. up to 70° C., while others can survive an acidity equivalent to *N.HCl*. Owing to their wide distribution, rapidity of growth and chemical versatility, bacteria and other micro-organisms profoundly affect the geographical pattern of life. By fixing nitrogen in the soil, they divert it from the atmosphere to the plant, and thence to the animal. By breaking down organic compounds returned to the soil, they maintain the circulation of biological elements. As inhabitants of the alimentary tracts of all animals, they aid in the digestion of cellulose and the synthesis of certain vitamins; as pathological agents, they are responsible for many of the infectious diseases; while, when exploited industrially, they provide man with the instruments for making many of his characteristic foods, and other useful products.

The Fabric of Life.—Unaided vision can discern objects down to about 0.2 mm. in diameter; the world revealed by the ordinary microscope ceases to be visible about 0.3 μ .; but the world of molecular and atomic structures explored by the chemist and physicist is not reached until the scale of significant distances has decreased to the order of a few Ångström units (Å). "The gulf between these two worlds may be gauged by the comparison that 1 μ . = 10,000 Å., and in this almost uncharted region lies the structural framework behind almost all cytological problems, including fundamental issues such as the physical basis of life" (Manton, 1942). The term *protoplasm*, applied by Schultze, in 1861, to denote the universal "living substance" of which organisms are made, has now been replaced by the more precise term *cytoplasm*, or cell material.

Cytoplasm is non-homogeneous, being functionally organised for respiration, energy storage and transfer, and the various chemical changes involved in growth, maintenance, secretion and decay.

According to Bensley (1942), cytoplasm includes : (1) structural units of a protein character that determine the architecture of the cell, (2) microscopic and sub-microscopic particles that modify special processes, and (3) cellular fluid or plasma.

These constituents may be separated by appropriate physical methods, such as the ultra-centrifuge, or explored directly by the electron-microscope, which, using a beam of electrons instead of light rays, can attain a magnification of 100,000 diameters, which is fifty times greater than that of the most powerful optical microscope.



Subdivisions.—Biochemistry may be classified according to the material examined and according to the purpose of the examination. The first subdivision includes plant biochemistry, animal biochemistry, human biochemistry, cytological chemistry, tissue chemistry, embryological chemistry, bacterial chemistry, enzyme chemistry, and the like. The second subdivision includes physiological, pathological, clinical, medical, industrial, analytical and

theoretical biochemistry. A more or less practical form of the science is now part of medical education, and is pursued partly for the information it gives about the normal working of the human machine, partly for the means it affords of detecting and measuring pathological conditions, and partly for the weapons it offers in the chemical warfare against disease and death.

Literature.—Biochemical research is in rapid progress throughout the civilised world, and the output of work is overwhelming. The main channels of information are scientific periodicals, reviews, textbooks and monographs.

Among the most important publications are: *The Biochemical Journal*, *The Journal of Biological Chemistry*, *Zeitschrift für physiologisches Chemie*, *Biochemisches Zeitschrift*, *Bulletin de la société de chimie biologique*, *Acta Phytochemica*, *Annual Review of Biochemistry*, *Annual Reports of the Chemical Society of Great Britain*, *Biological Abstracts*, *Biological Reviews*, *Nutrition Abstracts and Reviews*, *Chemical Reviews*, *Tabula Biologica*, *Chemical Abstracts* (British and American), *Ergebnisse der Physiologie*, *Symposia on Quantitative Biology*, *Enzymologia*, *Ergebnisse der Enzymforschung*, *Ergebnisse der Vitamin- und Hormonforschung*, *The Journal of General Physiology*, *Monographs on Biochemistry*, *Les Problèmes Biologique*. *Advances in Enzymology*. *The Harvey Lectures*.

Textbooks are dominated by the monumental *Biochemisches Handlexicon* of Abderhalden, and the *Handbuch der Biochemie* of Oppenheimer. In the English language, the most familiar are the books by Bodansky, Cameron, Cole, Gortner, Halliburton, Heilbrunn, Harrow and Sherwin, Hawk and Bergeim, Koch, McClendon, Mathews, Milroy, Morse, Parsons, Peters and Van Slyke, Plimmer, Pryde, Sumner, Thorpe, and Williams. A valuable survey of scientific books is *The Literature of Chemistry*, by E. J. Crane and A. M. Patterson.

Course of Study.—The plan most usually adopted in studying animal biochemistry is to start with the general composition of the organism, and the chemical characteristics of the chief organic and inorganic compounds that enter into its structure. Then, the chemical composition of the tissues and physiological systems is examined, and tissue properties are explained in terms of tissue chemistry. Finally, the composition of the diet is investigated, along with the significance of each constituent, its changes during digestion, and its intermediate metabolism and forms of excretion. Thus, starting with static, analytical biochemistry, the student proceeds to explore the innumerable and interlinked reactions that determine, in part, at least, the behaviour of "the thing called Man."

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ATOMIC WEIGHTS

Atomic number.	Name.	Sym- bol.	Atomic weight.	Atomic number.	Name.	Sym- bol.	Atomic weight.
1	Hydrogen .	H	1.0081	47	Silver .	Ag	107.880
2	Helium .	He	4.003	48	Cadmium .	Cd	112.41
3	Lithium .	Li	6.940	49	Indium .	In	114.76
4	Beryllium .	Be	9.02	50	Tin .	Sn	118.70
5	Boron .	B	10.82	51	Antimony .	Sb	121.76
6	Carbon .	C	12.01	52	Tellurium .	Te	127.61
7	Nitrogen .	N	14.008	53	Iodine .	I	126.92
8	Oxygen .	O	16.0000	54	Xenon .	Xe	131.3
9	Fluorine .	F	19.00	55	Cesium .	Cs	132.91
10	Neon .	Ne	20.183	56	Barium .	Ba	137.36
11	Sodium .	Na	22.997	57	Lanthanum .	La	138.92
12	Magnesium .	Mg	24.32	58	Cerium .	Ce	140.13
13	Aluminium .	Al	26.97	59	Praseodymium .	Pr	140.92
14	Silicon .	Si	28.06	60	Neodymium .	Nd	144.27
15	Phosphorus .	P	31.02	61	Illinium .	Il	—
16	Sulphur .	S	32.06	62	Samarium .	Sm	150.43
17	Chlorine .	Cl	35.457	63	Europium .	Eu	152.0
18	Argon .	A	39.944	64	Gadolinium .	Gd	156.9
19	Potassium .	K	39.098	65	Terbium .	Tb	159.2
20	Calcium .	Ca	40.08	66	Dysprosium .	Dy	162.46
21	Scandium .	Sc	45.10	67	Holmium .	Ho	163.5
22	Titanium .	Ti	47.90	68	Erbium .	Er	167.2
23	Vanadium .	V	50.95	69	Thulium .	Tm	169.4
24	Chromium .	Cr	52.01	70	Ytterbium .	Yb	173.04
25	Manganese .	Mn	54.93	71	Lutecium .	Lu	175.0
26	Iron .	Fe	55.84	72	Hafnium .	Hf	178.6
27	Cobalt .	Co	58.94	73	Tantalum .	Ta	180.88
28	Nickel .	Ni	58.69	74	Tungsten .	W	183.92
29	Copper .	Cu	63.57	75	Rhenium .	Re	186.31
30	Zinc .	Zn	65.38	76	Osmium .	Os	190.2
31	Gallium .	Ga	69.72	77	Iridium .	Ir	193.1
32	Germanium .	Ge	72.60	78	Platinum .	Pt	195.23
33	Arsenic .	As	74.91	79	Gold .	Au	197.2
34	Selenium .	Se	78.96	80	Mercury .	Hg	200.61
35	Bromine .	Br	79.916	81	Thallium .	Tl	204.39
36	Krypton .	Kr	83.7	82	Lead .	Pb	207.21
37	Rubidium .	Rb	85.48	83	Bismuth .	Bi	209.00
38	Strontium .	Sr	87.63	84	Polonium .	Po	—
39	Yttrium .	Y	88.92	85	—	—	—
40	Zirconium .	Zr	91.22	86	Radon .	Rn	222
41	Niobium .	Nb	92.91		(Emanation) .		
	(Columbium) .	(Cb)		87	—	—	—
42	Molybdenum .	Mo	95.95	88	Radium .	Ra	226.05
43	Masurium .	Ma	—	89	Actinium .	Ac	—
44	Ruthenium .	Ru	101.7	90	Thorium .	Th	232.12
45	Rhodium .	Rh	102.91	91	Protoactinium .	Pa	231
46	Palladium .	Pd	106.7	92	Uranium .	U	238.07

CHAPTER 2

BIOLOGICAL ELEMENTS

"This was the heavenly hiding-place
Wherein the spirit laughed a day.
All its proud ivories and fires
Shrunk to a shovelful of clay."

GEORGE RUSSELL.

BIOLOGICAL elements vary greatly in importance and in distribution. Some are present in all forms of life, others have been detected only in a few species. Some are present in large amounts, others occur as traces of doubtful significance.

CLASSIFICATION OF THE BIOLOGICAL ELEMENTS

Of the ninety-two elements believed to constitute the universe, at least sixty have been found by Vinogradov (1935) to be associated in one way or another with the fabric of life. From them are derived all the compounds and systems of biology, and, whether the organism be regarded as an episode in the history of matter or of spirit, its behaviour is restricted by the chemical properties of its constituents. Biological elements may be grouped (a) *chemically*, as metals and non-metals; (b) *physiologically*, as essential and non-essential elements; and, (c) *biologically*, as plant and animal constituents.

While these and similar classifications are useful for particular ends, it is simpler at the outset to take the elements in group order according to the periodic table, making a subdivision into *variable* and *invariable* elements, and recognising a quantitative distinction between *primary* elements, *secondary* elements, *micro-constituents*, and *contaminants*.

(1) **Invariable Primary Elements.**—*Hydrogen, Carbon, Nitrogen, Oxygen, Phosphorus.*—These make up the greater part of the organism, representing, individually, from 1 to 60 per cent. of the total weight. They are found in all known forms of life, and determine largely its physical structure.

(2) **Invariable Secondary Elements.**—*Calcium, Magnesium, Sodium, Potassium, Iron, Sulphur, Chlorine.*—These are equally necessary for life, but occur in much smaller quantities, usually from 0.05 to 1 per cent. of the total weight of the organism.

Among the vertebrates, calcium is a primary element.
In plants, sodium, calcium and iron are micro-constituents.

(3) **Invariable Micro-constituents.**—*Copper, Boron, Silicon, Manganese, Fluorine, Iodine.*—These are believed to be present in all forms of life. The concentrations are minute; usually less than 0.005 per cent. of tissue weight.

(4) **Variable Secondary Elements.**—*Zinc, Titanium, Vanadium, Bromine.*—These elements reach a relatively high concentration in certain species, but their occurrence and importance in other species are often doubtful.

(5) **Variable Micro-constituents.**—*Lithium, Rubidium, Caesium, Silver, Beryllium, Strontium, Cadmium, Germanium, Tin, Lead, Arsenic, Chromium, Cobalt, Nickel, Aluminium, Molybdenum, Barium.*—Some of these have only been detected in a few species, and their functions are obscure.

Micro-constituents regularly present, irrespective of known biological significance, are sometimes termed *trace elements*.

(6) **Contaminants.**—*Argon, Helium, Mercury, Thallium, Selenium, Bismuth and Gold.*—This classification (Fearon, 1933) includes thirty-nine elements; the additional twenty-one accepted by Vinogradov are mostly what he terms "ultra-micro" elements.

*Biological Elements classified according to their Distribution as
Percentage Body-weight of the Organism*

INVARIABLE (18).			VARIABLE (21).		
Primary (1 — 60%).	Secondary (0.05 — 1%).	Micro- constituents ($< 0.05\%$).	Secondary.	Micro- constituents.	Contaminants (?).
Hydrogen Carbon Nitrogen Oxygen Phosphorus	Sodium Magnesium Sulphur Chlorine Potassium Calcium Iron	Boron Fluorine Silicon Manganese Copper Iodine	Titanium Vanadium Zinc Bromine	Lithium Beryllium Aluminium Chromium Cobalt Nickel Germanium Arsenic Rubidium Strontium Molybdenum Silver Cadmium Tin Caesium Barium Lead	Helium Argon Selenium Gold Mercury Bismuth Thallium
5	7	6	4	17	7

Periodic Classification of the Biological Elements.—Chemical elements may be arranged in order of increasing atomic weight (a.w.) and atomic number (a.n.), the latter being the value of the nuclear charge as expressed by the number of satellite electrons carried by the atom. The nuclear charge increases with increase in atomic weight, and is indicated by the numerical position of the element in the periodic table. These fundamental properties are denoted by prefixes to the symbol for the element, the upper prefix representing atomic weight and the lower representing atomic number. As, for example, $^{14}_7\text{N}$ denotes nitrogen of a.w. 14 and a.n. 7. In symbols representing chemical compounds, the lower suffix always denotes the number of atoms present. Thus, O_2 represents the oxygen molecule, which is composed of two oxygen atoms.

Biological Elements classified according to Position in the Periodic Table

Group 0	Group I a b	Group II a b	Group III a b	Group IV a b	Group V a b	Group VI a b	Group VII a b	Group VIII a b
He 2	Li 3	Be 4	B 5	C 6	N 7	O 8	F 9	
Ne 10	Na 11	Mg 12	Al 13	Si 14	P 15	S 16	Cl 17	
Ar 18	K 19	Ca 20	Sc 21	Ti 22	V 23	Cr 24	Mn 25	Fe 26
		Zn 30	Ga 31	Ge 32	As 33	Se 34	Br 35	Cu 27 Ni 28
Kr 36	Rb 37	Sr 38	Y 39	Zr 40	Nb 41	Mo 42	Tc 43	Ru 44 Rh 45 Pd 46
		Cd 48	In 49	Sn 50	Sb 51	Te 52	I 53	
Xe 54	Cs 55	Ba 56	La 57	Hf 72	Ta 73	W 74	Re 75	Os 76 Ir 77 Pt 78
		Hg 80	Tl 81	Pb 82	Bi 83			
Rn 86		Ra 88	Pa 89	Th 90		U 92		

The periodic table can be divided into three regions by means of two lines. Invariable biological elements, with the exception of Be, are those above the upper line; variable elements occur between the lines; and ultra-micro and non-biological elements lie below the continuous line.

Isotopes.—Many elements occur in nature as mixtures of two or more closely related forms having the same atomic number and chemical properties, but differing slightly in atomic weight. Thus, chlorine (a.w. 35.3) is a mixture of about 75 per cent. ^{35}Cl and

25 per cent. ^{37}Cl , with a trace of ^{39}Cl . Hydrogen (a.w. 1.0078) is chiefly ^1H , with about 0.02 per cent. ^2H (*deuterium*). These related species are termed *isotopes*, and are defined as elements of similar atomic number but different atomic weight. Including the isotopic forms, over six hundred and fifty different species of atoms are now recognised as represented among the ninety known elements.

Isotopic forms of elements, such as ^{15}N and ^{32}P , are used to trace the course of metabolism in plants and animals (p. 47).

Functions of the Elements.—At least seven different functions may be ascribed to the biological elements :—

(1) *Plastic and Storage Elements.*—Carbon and Nitrogen, which form the framework of the tissue colloids, the *cytoskeleton* of the cell. Sulphur and Phosphorus, which form reactive groups in organic compounds.

(2) *Energy-exchange Elements.*—Hydrogen and Oxygen.

(3) *Skeletal Elements.*—Calcium, Magnesium, Phosphorus, Fluorine, Silicon.

(4) *Electrolytes and Osmotic Regulators.*—Cations of Sodium, Potassium, Calcium, Magnesium ; anions of Chlorine, Phosphate and Carbonate.

(5) *Catalytic Elements.*—Components of the oxidation-reduction systems : Iron, Copper, Manganese, Zinc, Iodine, Sulphur. Activators of enzymes : Calcium, Magnesium, Cobalt.

(6) *Replacement Elements.*—Members of which can supplement or substitute other elements.

(7) *Micro-constituents of Unknown Significance.*—Bromine, Nickel, Molybdenum, Silver, Arsenic, Silicon, together with the "ultra-micro" elements.

The chemical constitution of organisms is closely similar with regard to H, C, N, O, S and P, but the occurrence and content of elements such as Fe, Mn, Br, I, B, As, Ti, Va and Zn varies greatly from species to species. Vinogradov has shown that some organisms act as accumulators of specific elements, and thereby modify the surface composition of the earth. Starkenstein (1936) has pointed out that metals of group 5 (As, Sb, Bi) or of period IV (Hg, Pb, Th) are capable of causing chronic poisoning owing to their progressive retention by the organism.

Detection and Estimation of the Elements.—Elements of primary or secondary importance have been found and estimated by the routine procedure of chemical analysis applied to animal and plant ash.

The work has been greatly refined in recent years by the systematic use of the spectroscope (Ramage *et al.*, 1929–1936 ; Dutoit and Zbinden, 1929, 1930 ; Webb, 1937).

This enables traces of micro-constituents to be detected and photographed as line spectra.

THE ELEMENTS IN GROUP ORDER

Group I: { **Hydrogen.**
Alkaline metals: Lithium, Sodium, Potassium, Rubidium,
 Copper, Silver.

Hydrogen.—H, a.n. 1; a.w. 1.008. A primary element ranking next to oxygen and nitrogen in biological importance. The total H content of an organism depends largely on the water content. The adult human body contains approximately 9.9 per cent of hydrogen, chiefly as water.

Forms of Occurrence.—Free hydrogen, H_2 , is an end-product of cellulose fermentation, and may appear in the alimentary tract of herbivora, otherwise the free element does not appear to enter into the life-cycle of higher plants or animals. Ionic hydrogen, H^+ , is present in all aqueous solutions, and its concentration determines the degree of acidity. Ionisable hydrogen occurs in all acids and acid salts, of which the carbonates and the phosphates are of special biological importance. Water, $(H_2O)_n$, is the commonest natural form of hydrogen: it makes up 60–80 per cent. of most tissues, and 90–99 per cent. of secretions. Hydrogen peroxide, H_2O_2 , occurs as an intermediate reactant in tissue oxidations. Ammonia, NH_3 , may be included among the inorganic forms of hydrogen, but is more usually studied as a nitrogen derivative. Organic hydrogen is found chiefly in proteins and carbohydrates (7 per cent.), and in fats (12–14 per cent.). To a lesser extent it is present in all bio-organic compounds.

Significance.—Hydrogen is a primary constituent of organic foodstuffs, and constitutes the ultimate fuel of life, as well as possessing the greatest heat of combustion of all the elements. The H-ion, or **Proton**, is the most electro-positive and mobile of all ions. Its concentration qualifies the properties of many tissue constituents, notably colloids and colloidal systems, such as enzymes.

Deuterium.—A heavy isotope of hydrogen, 2H , was discovered in 1932, by H. M. Urey and his colleagues, and subsequently termed *deuterium*, D. Deuterium oxide, D_2O , occurs in all natural waters, the proportion being about 1:6,000 in rain water. This *heavy water* fraction can be concentrated by evaporation, and by fractional electrolysis H_2O and HDO can be removed.

	D_2O	H_2O
Melting point . . .	3.8°	0°
Boiling point . . .	101.42°	100°
Density at 25° . . .	1.105	1.0

When first discovered, it was claimed that pure D_2O is toxic to lower forms of life, and the suggestion was made that the physical changes of old age might be due to accumulation of heavy water in the organism. The alleged toxicity of D_2O has not been confirmed by later workers, and the organism appears to be indifferent to the compound in low concentrations. Thus, Erlenmeyer and Gärtner (1934) found that the animal body does not appreciably change the D_2O content of the water of the diet. By using compounds containing deuterium instead of hydrogen it has been possible to label molecules of fats, sterols and other biological compounds, and trace their fate in the organism (p. 47).

Lithium.—Li, a.n. 3; a.w. 6.94. A variable micro-constituent of plants, marine animals, human bone, muscle, and lung tissue. The metal resembles sodium and potassium in its pharmacological effects, but is much more toxic, 0.1–0.2 gm. per kg. body weight being fatal for dogs and cats. No specific function has yet been assigned to lithium.

In very few of the marine organisms examined by Webb (1937) was the lithium content higher than that of the environment; the value for sea water being 100–170 mg. Li per cubic metre (Bardet, *et al.*, 1937).

Sodium.—Na, a.n. 11; a.w. 23.00. Sodium is probably a universal micro-constituent in plants; the reported values range from 0.0075–0.15 per cent. of fresh tissue, marine species being richest. In animals the metal is found in much greater amounts, and makes up 0.1–0.5 per cent. of the total body weight of mammals. The concentration is always higher in the extracellular liquids (blood plasma, lymph, digestive secretions, sweat, urine) than in the cells. The value of human blood serum is kept remarkably constant at about 335 mg. Na per 100 ml., whereas the red corpuscles contain only about 0.016 per cent., or 7 mg. equivalents of Na^+ per litre.

Sea water contains 1.059 per cent. Na.

Sodium values for fresh human tissue, expressed in Na per cent., are: muscle, 0.064; kidney, 0.175; brain, 0.254; lung, 0.312; liver, 0.181; entire blood, 188 mg. per 100 ml.; spinal fluid, 290–465 mg. per 100 ml.; urine, 46–608 mg. per 100 ml.; bone, 0.2 per cent.

Among organs, the kidney is rich in sodium owing to its power of concentrating the metal during urinary secretion. Tissue cells other than those of the kidney usually have a sodium value below that of their liquid environment; transudates approximate to the sodium value of blood plasma; while true secretions, such as milk and urine, may show wide variations in their sodium content. The exceptionally high value for cartilage is explained by Bunge as being

the persistent affinity of this ancient tissue for the characteristic metal of the sea water, which at one time was the cradle of life.

The human excretion of sodium is about 20–30 gm. daily, expressed as NaCl; it comes largely from the condiments used in the diet, and is eliminated to the extent of 95–99 per cent., as a solute in the urine.

Significance.—Sodium is the chief circulating extra-cellular cation of the animal. Cell membranes, once formed, are relatively impermeable to Na^+ . The solubility of sodium salts favours their uptake, but restricts sodium storage, and the metal must be supplied continually in the animal diet.

Representative values for foodstuffs, expressed as mg. per 100 gm. fresh material are: meat, 65–80; milk, 43; eggs, 185; fish, 125; liver, brain, kidney, 110–160; cereals, 5–30; potatoes, 3–4; green vegetables, 3–15; root vegetables, 10–60; fruits, 0–3; nuts, 2–10.

McCance (1936, 1), from whose paper the above data are taken, observes that a man would have to eat twice his own weight of potatoes daily to obtain his biological requirement of sodium.

Herbivorous animals deprived of sodium develop a characteristic "salt hunger," attributed by Bunge to the disturbing effect of the excess of potassium present in all vegetables. Benedict (1915) has found 0.2 gm. NaCl *per diem* to represent the minimal requirement of adult man, although this quantity is exceeded ten or twentyfold to allow for loss of the metal by renal excretion.

Forced loss of sodium and chloride evoked in the human subject by an almost salt-free diet, aggravated by sweating, caused cramps, weakness, and severe cardio-respiratory distress on exertion (McCance, 1936, 2).

Special functions ascribed to sodium are:—(1) *A transport solute.* Since most sodium compounds are freely soluble in water, the metal may aid in the solution and distribution of metabolites. (2) *A physiological ion.* Na^+ , along with K^+ , Ca^{++} , Mg^{++} , H^+ and HO^- , is a member of a group of ions necessary for maintaining tissue excitability in higher plants and animals. (3) *An ionic-balance component.* Sodium provides most of the positively charged cations necessary to balance the various anions (Cl , HCO_3^- , HPO_4^-) present in body fluids, and thus help to maintain ionic neutrality.

The Na^+ and K^+ content of the blood are regulated by the adrenal cortex. Glandular dysfunction, as seen in Addison's disease, is marked by a characteristic fall in the plasma sodium, and when this is compensated by administration of about 10–20 gm. of additional NaCl *per diem*, the asthenia and vascular disturbances are rectified.

Land plants that have no extracellular circulation may contain almost no sodium, and it has been claimed that insects, such as

Drosophila, are independent of the metal to such an extent that they can lose 95 per cent. of their total Na without ill-effects.

Potassium.—K, a.n. 19; a.w. 39.10. A primary constituent of all plants; the concentration greatly exceeding that of sodium. Reported values range from approximately 0.1 to more than 1.0 per cent. K in fresh tissue, maximal concentration being found in the regions of active growth, such as leaf tips, secondary roots and pollen tubes.

Potassium is universal in animal tissues, and, unlike sodium, is associated chiefly with cellular structures rather than with fluids.

K⁺ is the chief ion within the red blood cell of the rabbit, monkey, and man; Na⁺ is the chief ion in the red cell of the cat and the dog.

Average values for fresh human tissues, expressed in K⁺ percentages, are: muscle, 0.349; kidney, 0.196; entire blood, 182 mg. per 100 ml.; serum, 19–24 mg. per 100 ml.; corpuscles, 428 mg. per 100 ml. Other values, in mgm. per 100 gm., are: human milk, 50; cow's milk, 150; potato (dry weight), 1.8; cereals (dry) 0.3–0.5.

Sea water contains about 0.38 gm. K per litre.

The total potassium content of higher land animals is usually slightly greater than the total sodium content, being 0.1–0.2 per cent. of the body weight. The internal distribution, however, of the elements is quite different. Potassium, maintaining a vegetable tradition, accumulates in the tissues; sodium, true to a marine ancestry, circulates in the fluids.

Forms of Occurrence.—By cold water extraction, plant tissues can be freed completely from potassium, which indicates that it occurs chiefly in ionic form.

After incineration of vegetable matter, potassium is found abundantly in the ash, almost entirely as the carbonate. After extraction by water and recrystallisation, this provides the "potash" of commerce, and, incidentally, the English name for the metal.

Baker's yeast contains about 4 mg. K per gm., of which 15 per cent. can be extracted by water. According to Conway, K⁺ in yeast can be replaced by NH₄⁺ without obvious disturbance of function.

Most of the potassium of higher animals can be extracted in the form of phosphate, but organic compounds are known. The wool fat of sheep is rich in lipide potassium, and is an important channel of excretion of the metal.

Significance.—(1) *A Photosynthetic Factor.*—In the plant, potassium is necessary for chlorophyll manufacture and function, although it appears to be absent from the chloroplast.

(2) *A Factor in Plant Growth.*—Potassium appears to be essential for normal cell division, nitrate reduction, and protein synthesis.

(3) *A Factor in Animal Growth*.—Miller (1923, 1926) reports that growth of young rats ceases when the potassium content of the dietary falls below 0.1 per cent.

(4) *A Physiological Ion*.—In the animal, potassium is the chief metallic ion of the cell interior, and forms one of the group necessary for tissue excitability. The passage of a nerve impulse along a fibre is accompanied by a temporary release of K ions.

(5) *A Diuretic*.—The mammalian kidney is more sensitive to potassium ions than to sodium ions, and potassium administration is followed by a compensatory diuresis.

(6) *A Radio-Active Element*.—Potassium is the most radio active of all biological elements. This is due entirely to the presence of an isotope ^{40}K , which constitutes about 0.01 per cent. of naturally occurring potassium, and which on disintegration yields calcium, beta particles and gamma radiation. The change is of interest because K^+ and Ca^{++} are physiologically antagonistic. Attempts to ascribe the unique biological properties of potassium to its radio-activity are not supported by the work of Glazko and Greenberg (1939), who found neither artificially prepared radio-sodium, ^{24}Na , nor radio-phosphorus could replace natural potassium in maintaining the beat of the isolated heart.

The European mixed dietary provides about 2–4 gm. K *per diem*, which is more than ample for nutritional requirements.

The general biochemistry of potassium has been reviewed by Vanhems (1934), and by Fenn (1940).

Copper.—Cu, a.n. 29; a.w. 63.57. An invariable micro-constituent of plants and animals. In plants the copper value is, roughly, one-fifth that of manganese and one-fifteenth that of iron. It ranges from 0.25 mg. Cu per litre of potato juice up to 40 mg. per kgm, in dried lettuce leaf. Leguminous plants and their seeds are rich in the metal.

Representative values, expressed in mg. Cu per kg. fresh edible material, are: almond, 12.1; apple, 0.8; asparagus, 1.4; bean, 6.5; cabbage, 0.5; carrot, 0.8; pea, 2.4; potato, 1.7.

In animals, oysters are exceptionally rich, the value being 24–60 mg. Cu per kg. fresh tissue. Red-blooded fish contain only 1–2 mg. per kg. Higher animals display copper-rich regions (liver, kidney, heart, brain, hair), and copper-poor regions (skin, lung, pancreas, spleen, skeletal muscle). Ramage and the Sheldons find that large stores of Cu accumulate in the foetal liver.

Values for fresh human tissue, in mg. Cu per kg., are: adult liver, 1.3–3.9; brain, 3.6–6.0; blood serum, 1.7; milk, 0.05–0.5 mg. per litre. The 70 kgm. adult human body includes 100–150 mg. Cu.

Copper is a primary element in the blood of some marine invertebrates, notably the king crab (*Limulus polyphemus*) and the lobster (*Palinurus vulgaris*), where it is part of the blue respiratory pigment, hæmocyanin, which is the analogue of the red pigment hæmoglobin, of higher animals. The copper content of sea water was formerly believed to be 100–200 mg. per cubic metre, but Atkins (1932) has shown that 10 mg. per cubic metre is more exact.

Forms of Occurrence.—At least four derivatives are known; *turacin*, a copper porphyrin found as a purple pigment in feathers; *hæmocuprin*, obtained from hæmocyanin; *hæmocuprein*, a copper-protein compound in red blood cells and serum; and *hepatocuprein*, found in liver (Mann and Keilin, 1938). Less than half the copper in plants occurs in a water-soluble form, which suggests the existence of other unidentified derivatives.

Significance.—(1) *A Respiratory Pigment.*—Copper as hæmocyanin partakes in oxygen transport in the blood of many arachnids, crustaceans, and molluscs.

(2) *A Feather Pigment.*—No special function has been found for the turacin of feathers. It is water-soluble, and may be a form in which the metal is excreted.

(3) *A Factor in Hæmoglobin Synthesis.*—Copper salts administered in micro-dosage are effective in raising the hæmoglobin content of mammalian blood in some conditions of anæmia. The metal is believed to aid in the synthesis of the porphyrin nucleus of hæmoglobin, and the mobilisation of stored iron.

(4) *A Factor in Plant Growth.*—In optimal minima, copper stimulates growth and germination of many plants, especially *Leguminosæ*. In higher concentrations, it is very toxic to all forms of plant life, especially *Algæ*.

(5) *A Factor in Animal Growth.*—Copper is a micro-essential in the nutrition of many animals, and when it is deficient in soil and pasture characteristic diseases occur among grazing animals.

(6) *An Oxidation Catalyst.*—Many oxidising enzymes in plants and some animal tissues are copper-protein complexes, such as *catechol oxidase*, from potato, *polyphenol oxidase*, from mushroom, and *ascorbic oxidase*. The wide distribution and probable importance of such catalysts explains the apparent universal need for copper as a micro-nutrient.

Dietary sources of copper range from 0.1 mg. Cu per kg. fresh vegetables to 44 mg. per kg. fresh calf liver. The mixed human diet provides 0.1 to 5 mg. Cu *per diem*. Milk is usually very deficient in Cu, and Daniels and Wright (1934) suggest that the diet of children of pre-school age should contain not less than 0.1 mg. Cu per kg. body weight.

Surveys of the copper content of food materials have been made by many workers, notably Lindow *et al.* (1929), and Cunningham (1931).

Copper as a Growth-inhibitor of Micro-organisms.—O'Meara and Macsween (1936) have made the observation that the failure of staphylococcus and other pathogenic organisms to grow in culture media may be due to the presence of copper, and recognition of this fact may necessitate a reclassification of bacteria, as many species are identified by their sensitivity to aerobic or other conditions, which in turn may be dependent on the presence of Cu ions.

Rubidium.—Rb, a.n. 37; a.w. 85.45. Sheldon and Ramage (1931) find rubidium as a constant micro-constituent in all human tissues, but its occurrence in lower animals and plants is very sporadic. The metal is closely related to potassium, and Rubenstein reports that marine diatoms and possibly some of the seed plants can replace Rb⁺ for K⁺ in cell growth. Higher animals cannot survive such substitutions. The rubidium content of sea water is assessed by Schmidt at 10–15 mg. per litre, a value that is almost certainly excessive in view of the fact that Ramage finds that marine animals never contain more Rb than 0.002 per cent. of their dry weight.

Silver.—Ag, a.n. 47; a.w. 107.88. Fox and Ramage (1931) find that silver has a wide but very sporadic distribution, maximal values being 0.2 per cent. of dry material in fungi, and 0.005 per cent. in the livers of marine lamellibranchs. It is a micro-constituent of the human body, being located chiefly in uterus, ovary and thyroid.

The silver content of sea water is about 20 mg. per cubic metre.

Cesium.—Cs, a.n. 55; a.w. 132.8. A doubtful constituent of the animal body. Sonstadt (1870) reported the presence of Cs in algae, mollusc shells and sea water, in which Thompson and Robinson (1932) subsequently failed to detect it, using a concentrate from 200 kg.

Group II: { Alkaline-earth metals: Magnesium, Calcium,
Strontium, Barium.
Zinc, Cadmium.

Magnesium.—Mg, a.n. 12; a.w. 24.32. The essential metal of chlorophyll, and, therefore, present in all green plants. It also occurs as a universal micro-constituent of lower plants.

Representative values, expressed in mg. Mg per 100 gm. fresh tissue, are: wheat grain, 129; spinach leaf, 76; carrot leaf, 62; carrot root, 19; potato tuber, 32; lentil, 45; cherry fruit, 20.

Some marine animals are very rich in magnesium, and the skeletons of echinoderms may contain upwards of 10 per cent. MgCO₃. In these organisms, Mg is being concentrated in preference to Ca, owing to the relatively greater supply available in the environment. The magnesium content of sea water is 0.136 per cent. the metal ranking next to sodium in quantitative importance.

Magnesium is universal in the skeleton, muscles and nerve tissue

of higher animals, as shown by the sensitive colorimetric methods now available for its estimation (Cruess-Callaghan, 1936). The distribution of the solute is very uneven, ranging from 2.7 mg. per 100 gm. of plasma to 23 mg. per 100 gm. of skeletal muscle, and up to 50 mg. per 100 gm. of human heart muscle. Magnesium and calcium are closely associated in the skeleton, but not proportionally distributed. Human bone has a magnesium phosphate content of 0.6 per cent. fresh tissue. Human blood serum has an almost constant value of 1-3.5 mg. per 100 ml., and red corpuscles contain 2.3-4 mg. per 100 ml. The urinary content varies widely from about 50 to 250 mg. per litre, the average being 110 mg., which corresponds to a daily intake of approximately 0.2 gm. of the metal, the residue being excreted by the intestine. The chief source in the diet is the chlorophyll of the vegetable food-stuffs. Human milk contains about 6 mg. per 100 ml.

Forms of Occurrence.—(1) Accompanying, supplementing, or replacing calcium as basic phosphate and carbonate in skeletal tissue.

(2) As a phosphate, in urinary precipitates of "earthy phosphates."

(3) As a porphyrin derivative in chlorophyll.

(4) As inositol hexaphosphate, or *phytate*, of Mg and Ca, which occurs in the outer coating of cereals and other seeds.

(5) As an unidentified micro-constituent of tissues and secretions.

Significance.—(1) *A Structural Element.*—Magnesium, like calcium, aids in forming the inorganic matrix of the animal skeleton.

(2) *A Transport Element.*—Magnesium aids in phosphate transport and excretion in animals.

(3) *A Physiological Ion.*— Mg^{++} and Ca^{++} are the chief divalent ions that regulate tissue excitability. The effect is complex. In muscle, they are complementary to a limited extent, and can antagonise K^+ . In nerve, Mg^{++} and Ca^{++} are antagonistic; Mg^{++} has a specific depressant action, partly neutralised by Ca^{++} . Intravenous injection of sufficient Mg to raise the level in the blood up to 0.02 per cent. results in deep anaesthesia and paralysis of the voluntary muscles, an effect that is immediately abolished by the injection of a corresponding amount of a soluble Ca salt.

(4) *An Enzyme Activator.*—Mg ions are necessary for the phosphatase enzymes of the acid type found in kidney, bone, intestine, and in the mammary gland and the blood plasma (p. 239); and the metal is necessary also for the activation of the glycolytic enzyme system of muscle, and for carboxylase systems.

(5) *A Factor in Plant Growth.*—In the absence of magnesium, green plants are unable to manufacture chlorophyll, and fail to grow. It is the catalytic metal in photosynthesis.

(6) *A Factor in Animal Growth.*—Rats and dogs on a diet deficient in magnesium develop epileptiform convulsions and die. The condition is specific, and independent of the calcium level in the blood, and, according to Brookfield (1934), is due to renal and hepatic dysfunction. These may arise from failure of the phosphatase enzyme systems owing to lack of the specific activator.

Calcium.—Ca, a.n. 20; a.w. 40.07. Calcium is invariably present in plants, and is essential for growth of all vegetation, with the possible exception of some lower forms. The content ranges from about 10 to 100 mg. Ca per 100 gm. fresh tissue. It is high in cereal grain.

Average values, in mg. Ca per 100 gm. fresh edible tissue, are: asparagus, 28; beetroot, 21; cabbage leaf, 119; lettuce leaf, 35; onion bulb, 42; potato tuber, 11; oat grain, 117; barley grain, 86; wheat grain, 90.

The values show marked differences depending on species and soil conditions.

In animals, calcium is universal, and occurs in skeletal and soft tissues.

The human body contains 2-2.5 per cent. Ca, 97 per cent. of which is located in the skeleton. Vertebrate bone contains about 10 per cent. Ca, recoverable as tricalcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, calcium carbonate, CaCO_3 , and calcium fluoride, CaF_2 . Fresh vertebrate tissue has a calcium value ranging from about 6 mg. Ca per 100 gm. in muscle, up to 20 mg. in kidney, and 34 mg. in thyroid. Milk is very rich; average values being 30-80 mg. for human milk, and up to 120 mg. Ca per 100 ml. for cow's milk. Consequently, milk and cheese are most important dietary sources of calcium. The output in human urine is very variable, ranging from 0.5 mg. to more than 50 mg. per 100 ml. The average for twenty-four hours is 200 mg., corresponding to a usual calcium intake of 1.0 gm. of the metal, 65-80 per cent. of which is excreted by the intestine.

Sherman emphasises the necessity for an adequate intake of calcium in the human diet, especially in the nutrition of children, and places the daily requirement at 0.75-1.0 gm.

Calcium appears to be an essential constituent of all tissues and tissue fluids in animals. It is concerned in the control of cell permeability and the stabilisation of the intracellular framework, or cytoskeleton. It is necessary for the working of the neuro-muscular system, the coagulation of blood in vertebrates, the clotting of milk, and the activation of some enzymes, notably pancreatic *lipase*.

Calcium is the fourth commonest element in the surface crust of the earth. "On account of its abundance, and its property for forming low-soluble carbonates and phosphates, calcium has been used in almost every phyla for the construction of internal and external skeletal structures" (Robertson, 1941).

Forms of Occurrence.—(1) *Insoluble Salts.*—Tricalcium phosphate, calcium carbonate, calcium fluoride. In man, 84–90 per cent. of bone calcium is present as phosphate; the rest is chiefly carbonate. Calcium fluoride is the characteristic salt of tooth enamel. Insoluble calcium soaps form much of the faecal calcium, especially when the diet is rich in fats.

(2) *Blood Calcium.*—Calcium is almost absent from the red blood cells, but exists in the serum in colloidal and non-colloidal forms. For human serum the average total value is 9–11 mg. Ca per 100 ml., about 6 mg. of which are non-colloidal and diffusible.

Significance.—(1) *A Regulator of Soil Acidity.*—Calcium carbonate is one of the natural factors concerned in reducing the acidity of soil, and is used for this purpose in agriculture.

(2) *A Plant Structural Constituent.*—Calcium occurs as a pectate in the cell walls of plants, especially fruits, and imparts rigidity.

(3) *An Animal Structural Constituent.*—Calcium is the characteristic metal of the animal skeleton, although it may be represented in part by magnesium and entirely by silicon in some lower forms of life. Skeletal tissue, in addition to its obvious mechanical functions, provides a vast but somewhat inaccessible reserve of calcium.

(4) *A Physiological Ion.*—In plants, Ca^{++} tends to antagonise the toxic effects of K^+ , Na^+ , and Mg^{++} . In animals, an increase in the concentration of Ca^{++} (or Mg^{++} , or H^+) within critical limits tends to depress neuro-motor excitability and nerve conductivity.

(5) *A Factor in Muscle Contraction.*— Ca^{++} in optimal concentration is necessary for muscle contraction. Excess causes increase in tone, leading to "calcium rigor." The effect is antagonised by K^+ , and appears to be independent of the general depressant action of Ca^{++} on the neuro-muscular junction.

(6) *A Factor in Blood Coagulation.*— Ca^{++} participates in the normal coagulation of shed blood. It can be replaced, partially, by the related metals, Mg, Sr and Ba. Given by mouth, calcium salts have no effect on coagulation time in normal subjects.

(7) *A Factor in Milk Coagulation.*—Caseinogen includes about 1 per cent. Ca. Under the influence of rennin, caseinogen is converted into a form that reacts with the other calcium salts of the milk serum, and gives rise to the insoluble casein. Hence, milk curd and cheese are relatively richer in calcium than the original milk.

Determinants of Calcium Metabolism.—In higher animals three factors operate: (1) *the parathyroid hormone, parathyrin*; (2) *the D vitamins*; (3) *sunlight or ultra-violet irradiation*. Parathyrin promotes phosphate excretions by the kidney, and releases calcium phosphate from the bones, thus indirectly raising the Ca level in

the blood. Parathyroid deficiency causes a fall in serum calcium until the lower level of 7 mg. Ca per 100 ml. is reached, when the syndrome of hypocalcæmia appears, characterised by tetany.

Vitamin D has little influence on the serum calcium level in health, but in conditions of hypocalcæmia it raises the calcium and phosphate content of the serum, and promotes intestinal absorption of phosphorus and calcium. Solar and ultra-violet irradiation act by enabling the organism to manufacture vitamin D from its sterol precursor, if already present in the tissues.

Calcium Metabolism in Man.—Dietary calcium may be (i.) absorbed into the blood stream as soluble calcium, (ii.) precipitated in the alimentary tract as calcium soap, (iii.) left unchanged as phosphate carbonate, and phytate.

Serum calcium may be (i.) precipitated as skeletal calcium, (ii.) excreted as urinary calcium, (iii.) reabsorbed into the intestine.

"There can be no doubt that the development of satisfactory methods of eliminating Ca and Fe with insoluble salts must have been essential for evolutionary survival. Thus, loss of shell is a frequent event in the evolution of some invertebrate phyla, and must have thrown a heavy strain on the excretory mechanism. . . . Man would not have evolved had not the early mammals acquired the property of excreting unwanted calcium in an insoluble form by the gut, where it could not cause mechanical obstruction" (McCance, 1936, 1).

Skeletal calcium exists in the form of the complex salt, $\text{CaCO}_3 \cdot 3\text{Ca}_3(\text{PO}_4)_2$, and is not in simple equilibrium with the serum calcium. Calcification is a process involving the coaction of several factors, one being the phosphatase enzyme present in plasma and cartilage, another being the maintenance of the optimal calcium-phosphate ratio in the plasma, which normally is such that $[\text{Ca}] \times [\text{PO}_4] = 36$, measured in mg. Ca and P per 100 ml. of blood.

Strontium.—Sr, a.n. 38; a.w. 87.63. Fox and Ramage were the first to show that strontium is generally distributed in marine animals, and their work has been confirmed by Webb. Strontium is the fifth most abundant metal in sea water, the concentration being about 13.5 mg. per litre.

Strontium has also been found in the ash of marine plants, and as a micro-constituent in the animal skeleton, the liver and the lung of the human foetus. Its chief location appears to be the retina.

Barium.—Ba, a.n. 56; a.w. 137.4. Owing to the low solubility of barium sulphate, the maximum concentration of Ba ions in sea water is 0.48 mg. per cubic metre. The metal was detected in foraminifera by Schultze, and has been found by Webb in about half the marine organisms examined (1937). Ramage and Sheldon report its presence

in the choroids of cattle, but not in those of a wide selection of other vertebrates.

Barium salts are toxic to the higher animal, and the metal was unexpectedly found in the Brazil nut by Seaber (1933) during an investigation of an obscure outbreak of food poisoning.

The metal is concentrated in the husk, or inner part of the testa, where it makes up 8 per cent. of the total ash. Barium has several times been found in the ash of terrestrial plants, and may substitute other alkaline earth metals.

Zinc.—Zn, a.n. 30; a.w. 65-37. A universal micro-constituent. Among plants, values range from less than 1 mg. Zn per kg. fresh tissue in fruit pulp, up to 10 mg. per kgm. in leaves rich in chlorophyll, such as lettuce, cress, spinach, dandelion. Marine animals show values ranging from 3.5 mg. per kg. in elasmobranchs up to 188.5-341 mg. in oysters. About half of the total zinc in oysters can be removed as a simple solute, by dialysis. The 70 kg. human body contains on an average 2.2 gm. of zinc, most of which is in bone and hair. The ordinary mixed diet supplies about 12 mg. Zn daily.

Values, in terms of mg. per kg. fresh tissue, are: liver, 10-76; brain, 8-10; muscle, 5-50; blood serum, 4; red cells, 8 (all of which is in carbonic anhydrase). Thyroid and ovary are specially rich. The metal is constantly present in human and cow's milk, in egg yolk, and in some snake venoms.

The zinc content of sea water is 2-60 mg. per cubic meter.

Significance.—Zinc in low concentration stimulates plant growth, especially that of fungi and cereals. In higher concentration, over 1:100,000 ZnSO_4 , it is very toxic to most plants, and can inhibit completely sugar fermentation by yeast.

"Mottled-leaf" disease in fruit trees has been traced to zinc deficiency in the soil.

(1) Stirn, Elvehjem, and Hart (1935), and Bertrand and Bhattacharjee (1935) have shown that rodents fed on a diet deficient in zinc, though adequate in other respects, do not grow to maturity.

(2) It has been shown that, if zinc is present, the hypoglycæmic action of protamine-insulin or spermine-insulin compounds is greatly prolonged. Insulin preparations often contain zinc as a micro-constituent.

(3) The enzyme *carbonic anhydrase* (pp. 63, 253) contains 0.33 per cent. of zinc, and is the only biological compound of the metal identified as yet. Its existence shows that the zinc must be an essential micro-requirement in the animal diet.

(4) Bertrand and Vladesco have shown that the accessory glands of the male genital apparatus in mammals are rich in zinc, and the

seminal fluid even richer, and they suggest that the metal may play a part in fertilisation.

(5) Delezenne (1919) held that the power of snake venom to bring about rapid hydrolysis of nucleoproteins and phosphatides was correlated with its large zinc content. Cristol (1923) was led to conclude that this property of zinc enabled it to act as a necessary catalytic agent for the chemical changes that take place at mitosis. He observed that in malignant tumours or in the blood and liver in cases of leucocythæmia—both tissues in which mitosis is proceeding very rapidly—the zinc content was from 3 to 18 times the normal. Cruickshank (1936) has surveyed the sources and distribution of zinc in relation to human nutrition, and claims that tuberculosis is associated with a zinc deficiency, and malignant conditions with chronic zinc poisoning.

Cadmium.—Cd, a.n. 48; a.w. 112.4. This metal is usually associated in nature with zinc, and has been detected spectroscopically only in the liver of marine molluscs. Its significance is unknown.

Group III: Boron, Aluminium.

Boron.—B, a.n. 5; a.w. 11. This non-metal has been detected in all plants examined. The value is least in cereals (the ash of which contains about 0.5 gm. boric acid, H_2BO_3 , per kg.), high in the beetroot, and maximal in the date fruit (30 mg. B per kg. pulp). Grape vines and wine are rich in boron.

Sea water contains about 4.5 mg. B per litre. Borates must form an important part of the buffer mechanism of sea water, being second only to carbonates, and exceeding the combined effects of phosphates, arsenates and silicates. Webb finds B constant in marine organisms, the value being approximately that of the environment. There is little tendency to accumulate or exclude the element. Its concentration in higher animals is low and uncertain; the mammalian value being about 0.01 mg. B per kg. fresh tissue, or less. It is not a normal micro-constituent of milk, but may be introduced as a preservative.

Significance.—Boron is necessary for the development of higher plants, and appears to be associated with carbohydrate translocation (Dennis, 1937). In concentrations above 1:100,000 it retards growth. Fungi and certain green algae are extremely inert towards boron, and the toxic action of the element may be used to differentiate them from the relatively susceptible bacteria. The absence of boron from milk suggests that the element is not essential for the higher animal. Given in small doses for long periods it has no obvious effect on growth or metabolism. Large doses are toxic, and cause gastro-intestinal disturbance, inhibition of dermal secretion, and loss of hair.

Aluminium.—Al, a.n. 13; a.w. 27.1. This metal, common as aluminium silicate on the surface of the earth, is relatively rare in the organism.

It is present in all plants, being maximal in some mosses, which, according to Vinogradov, act as Al accumulators.

The value for fresh vegetable foods ranges from 35 mg. Al per kg., in cherries, and 40 mg. in onions, down to less than 1 mg. in apples and oranges. Green leafy vegetables have a value of 10–20 mg. per kg.

Aluminium is an occasional if not a frequent micro-constituent of animals.

Values reported are: dog's blood, 0.23 mg. per 100 ml.; human liver, 0.1–1.2 mg. per 100 gm.; human kidney, 0.13–0.87 mg. per 100 gm. Lung tissue, especially from older animals, is relatively rich in the metal.

After oxygen and silicon, aluminium is the most abundant element in the earth's crust, and all clay, dust and detritus is liable to introduce both Al and Si as contaminants into the organism. McCollum has shown that the skin, lungs and intestinal mucosa contain at least twice as much Al as the other organs, which as a rule have an Al value of less than 0.5 parts per million of fresh tissue.

The value for sea water is provisionally assessed at 0.6–2.4 mg. Al per litre.

Significance.—Considering its natural abundance, the very low concentration of aluminium in living organisms is remarkable, and, in consequence, the metal is not regarded as necessary for growth or function. There is no conclusive evidence that small doses of aluminium over long periods have any effect on the growth or health of higher animals, but the subject is still disputed. The lethal dose of the sulphate or the chloride for rats, rabbits, and guinea-pigs is 5–8 gm. per kg. body weight, injected subcutaneously. Resulting changes are degeneration of the renal tubules, necrosis and liver atrophy. Spira (1933) believes that chronic poisoning is liable to occur owing to the careless use of aluminium cooking utensils, but this is deprecated by Monier-Williams (1935).

Group IV: { Carbon, Silicon, Titanium.
Germanium, Tin, Lead.

Carbon.—C, a.n. 6; a.w. 12.

The twelfth element in order of abundance on the surface of the earth. It occurs chiefly in oxidised forms, as CO_2 in the atmosphere, H_2CO_3 in the ocean and other waters, and as insoluble carbonates in rocks and soil.

Carbon is the characteristic element of organic material, and accounts for 15–20 per cent. of the total weight of higher organisms. Englia has estimated that about one-thousandth part of the carbon of the earth's surface is in biological use. It occurs, united to hydrogen, in all bio-organic compounds, notably, the three great families of lipides, carbohydrates, and proteins.

Characteristics of Carbon.—The dominant position of carbon in the fabric of life is due to unique chemical properties having biochemical consequences, and it has even been suggested by Jeans

that life organisation is a latent property of carbon, which is *biogenic* in the sense that iron is magnetic or radium is radio-active.

(1) *Electro-chemical Character*.—Carbon comes midway in the periodic table, and has the property of uniting with all other known elements (except the members of the argon group). It is able to form stable compounds with strongly electro-positive elements, such as hydrogen, and with strongly electro-negative elements, such as chlorine.

(2) *Tetravalency*.—A carbon atom can unite with four monovalent elements or their equivalent.

(3) *Mutual Combination*.—Carbon atoms freely unite with each other in various ways :—

By single-bond linkage, as in *ethane*, $\text{H}_3\text{C}—\text{CH}_3$;

By double-bond linkage, as in *ethylene*, $\text{H}_2\text{C}=\text{CH}_2$;

By triple-bond linkage, as in *acetylene*, $\text{HC}\equiv\text{CH}$.

Double and triple-bond linkages are said to be *unsaturated*, and are less stable than single-bond linkages.

(4) *Chain Formation*.—Carbon atoms may unite to form straight, open, or linear chains ; and closed, cyclic chains, or rings.

(5) *Group Formation*.—Individual grouping tends to persist in organic molecules ; and *radicles* or particular groups of atoms may retain their identity through a series of reactions affecting other parts of the molecule.

These and other properties make possible the existence of a vast community of carbon compounds, which displays :—

(a) *Variety*.—At least a third of a million organic compounds are known.

(b) *Complexity of structure* unrivalled among inorganic compounds.

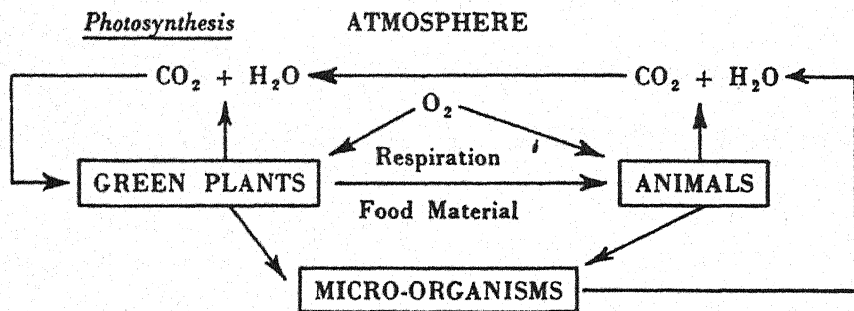
(c) *Family characteristics* due to presence of specific radicles.

(d) *Type reactions*, by means of which particular groups can be identified.

(e) *Isomerism*.—Organic compounds may be so complex that a simple molecular formula is insufficient to distinguish them. For example, $\text{C}_3\text{H}_6\text{O}$ may represent acetone ($\text{CH}_3\cdot\text{CO}\cdot\text{CH}_3$), acetaldehyde ($\text{CH}_3\cdot\text{CH}_2\cdot\text{CHO}$), or allyl alcohol ($\text{CH}_2 : \text{CH}\cdot\text{CH}_2\cdot\text{OH}$). These compounds are *isomers* in that they contain similar sets of atoms arranged differently. Isomerism is a feature of the great family of carbohydrates.

The Carbon Cycle.—Carbon occurs fully oxidised as CO_2 in the lower atmosphere to the extent of 0.04 per cent. by volume, and as H_2CO_3 in all ocean and fresh waters. It circulates freely in the biosphere, being accepted as CO_2 , and subsequently reduced to organic compounds, by the photo-synthetic mechanism present in

all green plants, where it is stored or transferred as food material to animals and to the flora and fauna of the soil or the sea. By the respiratory processes, necessary for the maintenance of all forms of life, organic compounds are eventually oxidised to H_2O and to CO_2 , which is restored thus to the environment.



The Carbon Cycle

Silicon.—Si, a.n. 14; a.w. 28.3. The most abundant element in nature, next to oxygen. Aluminium silicate is the chief constituent of clay, and silicates as a class make up 55 per cent. of the earth's crust. Silicon is universal in plants, especially the stems of cereals, bamboo, and coarser grasses. Expressed as percentage SiO_2 in total ash, representative values are: wheat, 2.28; barley, 22.3; oats, 42.64. Potato tuber ash contains about 2 per cent., the bulk being in the skin. Silica, SiO_2 , is the characteristic structural component in the skeletons of silicious sponges. The Si content of sea water is very variable, and ranges from 40 mg. up to 2,000 mg. per cubic metre, the higher values being obtained in shallow water containing suspended silicate.

In higher animals, it is a universal micro-constituent, especially in connective tissue. Average values range from 0.1–0.35 mg. SiO_2 per gm. fresh material. Silicotic human lung may have a value as high as 123 mg. per 100 gm. dry tissue.

Forms of Occurrence.—Skeletal silicon is found as an opaline silicate. Vegetable silicon appears to be a derivative of cellulose or other polysaccharides. In higher animals, the only compound identified is a sterol silicate found in bird's feathers.

Significance.—(1) *A Structural Element.*—Silica confers rigidity to the stems of cereals and grasses, but other factors also assist. It is the supporting element in the skeletal tissue of unicellular marine organisms, and may serve a special purpose in photosynthesis on

account of its great transparency. The association of silica with connective and scar tissue has led to its application in the treatment of diseases. The association is also seen in *silicosis*, a characteristic fibrosis of the lung due to inhalation of silica dust (King and Belt, 1938).

(2) *An Anti-Parasite*.—There is some evidence that silica aids in the protection of cereals against fungi, rust, and other infections.

(3) *A Replacement Element*.—Silicon is a non-metal closely related to carbon, and silico-organic compounds might be expected to appear in place of some carbon compounds, especially in plants.

Titanium.—Ti, a.n. 22; a.w. 46.1. An obscure micro-constituent of plants and higher animals. Fish, including mackerel, carp, herring, whiting, have values from 0.3 mg. Ti to 0.9 mg. per kg. fresh tissue. Mammalian liver has an average value of 0.6 mg. per kg. The metal has been detected spectroscopically in human lung tissue and blood ash. Webb has shown that serious errors may arise from the use of carbon electrodes contaminated by traces of titanium which are unmasked by the alkalis in tissue ash. For this reason, many claims as to the distribution of titanium require confirmation.

Germanium.—Ge, a.n. 32; a.w. 72.5. Traces of this rare metal have been detected in the ash of some seaweeds, and in blood. Steinberg (1939) claims that traces of Ge are necessary for growth of common moulds.

Tin.—Sn, a.n. 50; a.w. 118.7. Tin has been detected in most tissues of the higher animal, especially human brain, spleen, and thyroid. The amount present is very small and may be due to food contamination.

The most reliable estimations of tin in organisms are the analyses of Orton (1924), who found from 10 to 55 parts per million of fresh weight in oysters (the higher figure only for green specimens), and of Bertrand and Ciurea (1931), who found 0.4 to 4 parts in most mammalian organs, but up to 26 parts in the lingual mucosa.

Lead.—Pb, a.n. 82; a.w. 207. Traces of lead occur in plants, especially grasses, grown on plumbiferous soils. It is present in many marine animals, especially corals, crustacea, and molluscs, and is an erratic micro-constituent of higher animals, appearing inconsistently in human tissues in a manner that suggests lack of specific physiological function (Minot, 1938).

Group V : { **Vanadium.**
 { **Nitrogen, Phosphorus, Arsenic.**

Vanadium.—V, a.n. 23; a.w. 51. This rare metal is a characteristic constituent of the blood of some marine animals (holothurians and ascidians), where its significance is unknown. It has been found in timber-ash, and as a micro-constituent of animal tissues, and tends to accumulate in the liver, where it may promote oxidation of phospholipides (Bernheim).

Nitrogen.—N, a.n. 7; a.w. 14.01. Nitrogen has a very low affinity for other elements, and consequently most of the nitrogen

of the environment occurs in a free state in the atmosphere. Inorganic nitrogen as nitrate is widely distributed on the surface of the earth, and forms vast deposits in arid regions, as in Chile.

Nitrogen occurs in all living organisms, mostly as a constituent of protein, and is found in substances of organic origin in soil and sea water.

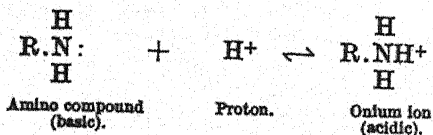
Forms of Occurrence.—The chief biochemical compounds of nitrogen are :—

A. *Inorganic* : NH_3 , NH_2OH (hydroxylamine), HNO_2 , HNO_3 , and their salts.

B. *Simple organic* : HCN , HO.CN (cyanic acid), HS.CN (thiocyanic acid), CON_2H_4 (urea), R.NH_2 (amines), R.CO.NH_2 (amides), $\text{R(NH}_2\text{).COOH}$ (amino acids), R.NH.CO.NH_2 (ureides), R.NH.C(NH).NH_2 (guanidines).

C. *Complex organic* : purines, pyrimidines, porphyrins, proteins, etc.

Many important biological properties reside in the amino group, NH_2 . Nitrogen is trivalent, and when these valencies are satisfied still possesses a "lone pair" of orbital electrons which are able to accept a proton, or H^+ -ion, thus conferring a positive charge on the group and making it a potential H^+ -ion donator.



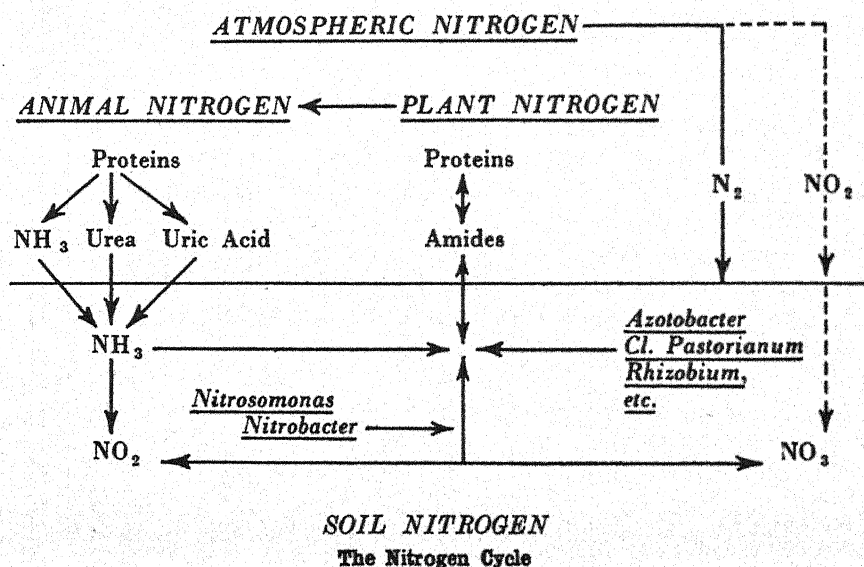
The Nitrogen Cycle.—Nitrogen leaves the atmosphere both directly and as oxides of nitrogen. The oxides are formed in the upper reaches of the atmosphere by photo- and electro-oxidation, and, dissolved in rain, reach the sea and soil as nitrous and nitric acids.

Free atmospheric nitrogen is assimilated by soil organisms, and by root-nodules of leguminous plants (peas, beans, and clover). Thus are made available for life several highly reactive forms of nitrogen, ranging from the strong acid, HNO_3 , to the base, NH_3 . From these, the plant synthesises proteins and other nitrogen compounds which may be transmitted to the animal, or back again to the soil. The higher animal excretes its waste nitrogen as urea, which is rapidly attacked by micro-organisms, and converted into ammonia, thus enabling the nitrogen to continue in circulation. Among birds and reptiles, waste nitrogen is excreted as purines, principally uric acid, which is a much more stable and less soluble compound than urea. By this process, nitrogen has

been temporarily diverted to form the great guano deposits of the Pacific Islands.

Absorption of atmospheric nitrogen by leguminous plants, with consequent enrichment of the soil, was demonstrated, in 1838, by Boussingault, and shown to be located in the root-nodules, by Hellriegel, in 1886. From these nodules, Beijerinck, in 1888, isolated a nitrogen-fixing organism, *B. radiculicola*, now termed *Rhizobium*, which, however, seems incapable of operating when apart from the host plant. Nitrogen-fixing nodules occur in a few higher plants, such as the roots of alder trees and the leaves of *Rubiacea*. Atmospheric nitrogen is also assimilated by soil organisms, notably by the anaerobe *Clostridium pastorianus* (Vinoogradsky, 1893), and the aerobe *Azotobacter* (Beijerinck, 1894), which occurs in all fertile soils down to a depth of about 50 cm. *Clostridium* occurs at a lower depth, and also is found in fresh and salt waters. *Radiobacter*, which accompanies *azotobacter*, has the power of oxidising N_2 into HNO_2 and HNO_3 , which, in turn, can be reduced to NH_3 .

The elements Ca (or Sr), Mg, Fe and Mo (or Va) are necessary soil constituents for the biological fixation of nitrogen. Ammonia, derived from the decomposition of nitrogenous compounds such as proteins and urea, is oxidised to HNO_2 by the soil organism *Nitrosomonas*, and subsequently oxidised to HNO_3 by *Nitrobacter*.



Phosphorus.—P, a.n. 15; a.w. 31.04. An essential constituent of all plants, the normal range being from 0.1–0.8 per cent. of dry tissue. The value is usually less than that for calcium, but more than that for magnesium or sulphur. The phosphorus content tends to increase with progress up the evolutionary scale. The adult human body contains about 1 per cent., or, approximately, 700 gm. of phosphorus. Of this, 600 gm. are in the skeleton, 57 gm. in muscle, 5 gm. in nerve tissue, and 2 gm. in the blood. The phosphorus content of milk varies greatly with species of animal, and rate of formation of the young skeleton; average values are: human milk, 0.05; cow's milk, 0.18; expressed as percentage phosphoric acid. The daily output of the human adult varies from 0.3 gm. P to 2.0 gm., two-thirds of this is excreted by the kidney. The value for inorganic urinary phosphorus ranges, in man, from about 30 to 200 mg. per 100 ml., the average being 80 to 90 mg. Organic urinary phosphorus ranges from 0.7 to 18 mg., the average being 6 mg., representing 19 mg. H_3PO_4 .

Forms of Occurrence.—(1) *Phosphoric Acid* (ortho phosphoric acid), H_3PO_4 , provides the ions H_2PO_4^- and HPO_4^{2-} of tissues and tissue fluids, and the PO_4^{3-} of bone.

(2) *Pyrophosphoric acid*, $\text{H}_4\text{P}_2\text{O}_7$, forms a series of esters of high chemical-bond energy that act as energy carriers in muscle contraction, carbohydrate metabolism, and many other biological events.

Significance.—Phosphates act as the transport agents, and by forming labile esters, controlled by the phosphatase enzymes, they convey glycerol and simple sugars from the intestine to the blood, transfer calcium to and from the skeleton, carry lipides to the tissues, and participate in the carbohydrate metabolism of muscle, liver and other organs.

(1) *Structural Phosphate*, including phytate, in plants, and bone salts, in animals.

(2) *Labile Phosphate of Carbohydrate Metabolism.*—Hexose phosphates formed during sugar degradation or polymerisation.

(3) *Labile Phosphate of Lipide Metabolism.*—This participates in transport and degradation of aliphatic acids.

(4) *Labile Phosphate of Muscular Contraction.*—Phosphagens, including phosphocreatine, phosphoarginine and adenosine triphosphate. These contain energy-rich phosphate bonds.

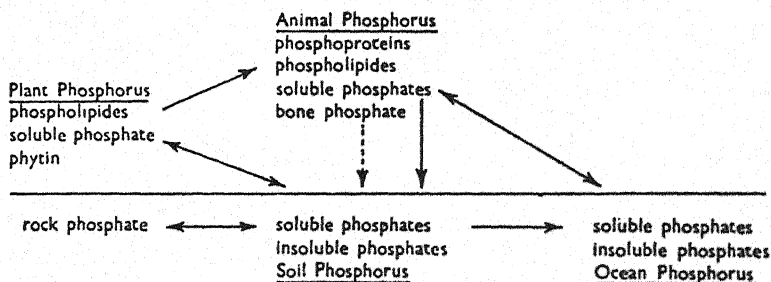
(5) *Lipide Phosphorus* of cell architecture.

(6) *Nuclear Phosphorus.*—The nucleoproteins that form the framework of chromatin and the carriers of heredity factors.

(7) *Buffer Phosphates*, stabilising the H-ion concentration of cells and tissue fluids.

(8) *Phosphoproteins*, such as casein, provide the chief source of phosphorus for young mammals.

The Phosphate Cycle.—Phosphorus circulates in the kingdoms of life in the oxidised form of phosphate. Plants elaborate soil phosphate into phospholipides and other phospho-compounds, which are transformed by the animal into phosphoproteins and tissue phosphates, the residue being returned to the soil or the sea as free phosphate. Soil phosphate is one of the important limiting factors in plant growth, and its provision is one of the tasks of applied agriculture.



The phosphate content of sea water is very variable, and ranges from almost zero, on the surface, to 0.004 per cent., expressed as PO_4 .

In higher animals, phosphate is excreted chiefly by the kidney, distribution being regulated by the parathyroid gland and the vitamins of the D group (pp. 197; 274). Phosphate removal or retention affects the calcium phosphate reserves of the skeleton, and thus indirectly controls calcium metabolism.

Arsenic.—As, a.n. 33; a.w. 75. Salts of arsenic are highly poisonous, and the metal is chiefly of toxicological interest. Gautier and Bertrand have shown that As is a universal micro-contaminant of plants, animals and their terrestrial or aqueous environment. Vernadsky reports concentrations in the earth's crust of the order of 0.11 parts per million (p.p.m.).

Arsenic occurs in sea water to the extent of about 11 to 20 mg. As per cubic metre, and has frequently been included in phosphate estimations by mistake (Atkins, 1927). Marine crustacea and some fish are notably rich, values for the soft tissues, expressed in p.p.m., are: plaice, 3; oyster, 3-10; lobster, 36-110; prawn, 40-174; shrimp, 12-40; mussel, 40-119 (Chapman, 1926). Cox (1925) notes that the urine of fish-eaters may contain up to 0.58 mg. As_2O_3 per litre, "a level pre-

viciously only associated with chronic As poisoning." The absence of harmful effects may be because much of the As is in an organic non-ionisable form, although it is possible that some examples of poisoning by shell-fish, ascribed vaguely to bacterial contamination, are really due to As.

In mammals, the distribution is so minute and variable that it can have little biological significance. Impure table salt is a source of arsenic in human tissues. The metal tends to accumulate in skin and hair.

Group VI : { Chromium, Molybdenum.
Oxygen, Sulphur, Selenium.

Chromium.—Cr, a.n. 24 ; a.w. 52.0. Traces of this metal have been detected spectroscopically in mammalian blood and tissue ash, especially thyroid and spleen, but, as Webb points out, these data, when obtained by the use of graphite electrodes, are open to question. Chromium salts are very injurious to the kidney, and the biological occurrence of the metal is probably adventitious.

Selenium. Se, a.n. 34 ; a.w. 78.9. A disease of farm animals may arise from traces of Se in plants, and a derivative has been isolated (Horn and Jones, 1940).

Molybdenum.—Mo, a.n. 42 ; a.w. 96. According to ter Meulen (1931) Mo occurs in nearly all tissues, the maximum being 9 parts per million of dry weight in plants, and 1.5 in animals. His spectrographic technique has been adversely criticised by Dingwall and others (1934), who find that the Mo content of plant tissues depends entirely on the environmental supply. Mo may be necessary for nitrogen-fixation in the soil.

Oxygen.—O, a.n. 8 ; a.w. 16.00. The most abundant and widely distributed of all elements. It constitutes about one-half of the earth's crust, and about two-thirds of plant and animal tissues. It is an essential element in almost every bio-organic compound, and is necessary, directly or indirectly, for the respiration that accompanies all life. Higher organisms are *aerobic*, and need a continual supply of free, molecular oxygen, although some, when compelled, can go into a temporary state of "oxygen-debt."

Some lower organisms, chiefly bacteria, are *anaerobic*, and can only utilise oxygen in combined forms. A few, the *obligatory anaerobes*, are poisoned by free oxygen.

Significance.—Oxygen is unique in being the only element assimilated in free, molecular form by the animal. Within the organism, it serves to liberate energy. The majority of chemical processes in life are oxidations, and the work performed is derived ultimately from energy obtained by oxidation.

Sulphur.—S, a.n. 16 ; a.w. 32.06. In plants, sulphur is universal and fairly uniformly distributed as proteins, organic sulphides, and inorganic sulphates. Values range from about 0.01 to 0.2 per

cent. of fresh material. In higher animals, the sulphur value depends largely on the amount of scleroprotein present. Thus, mammalian muscle has about 0.2 per cent. total sulphur; skin, hair, feathers, and other epidermal structures may have up to ten times as much. The sulphur content of foods has been surveyed by Masters and McCance (1939).

The total sulphur of human urine ranges, normally, from 30 to 300 mg. per 100 ml., according to the diet, the average being about 90 mg., expressed as H_2SO_4 .

Forms of Occurrence.—(1) *Inorganic Sulphates.*—Ions of H_2SO_4 occur in almost all plant and animal tissues. They are a form in which sulphur is distributed and excreted.

(2) *Inorganic Sulphides.*— H_2S and alkaline sulphides are products of bacterial metabolism, and may arise in the alimentary tract of the higher animal. They are readily oxidised to sulphates after absorption.

(3) *Organic Sulphates.*—Esters of phenols, cresols, indoxyl, and other alcohols. They appear as detoxication products in mammalian urine. *Taurine* occurs in bile acid.

(4) *Organic Sulphides.*—Allyl sulphide, or "oil of garlic," and ethyl sulphide occur in many *Liliaceæ*, especially onion and garlic.

(5) *Thiocyanates.*—Potassium thiocyanate, KSCN , is a frequent but obscure micro-constituent of human saliva, bile, and urine. Organic isothiocyanates, or "mustard oils," occur as glucosides in many plants.

(6) *Thio-amino acids*, cystine, cysteine, methionine.

(7) *Thio-peptides*, glutathione.

(8) *Thiols*, thioneine.

(9) *Thiamine*, or vitamin B_1 .

(10) *Sulpholipides.*—Fat-like sulphur compounds found in brain.

(11) *Mucositin*, or glucothionic acid, found in glycoproteins.

(12) *Chondroitin sulphate*, found in skeletal tissue of animals.

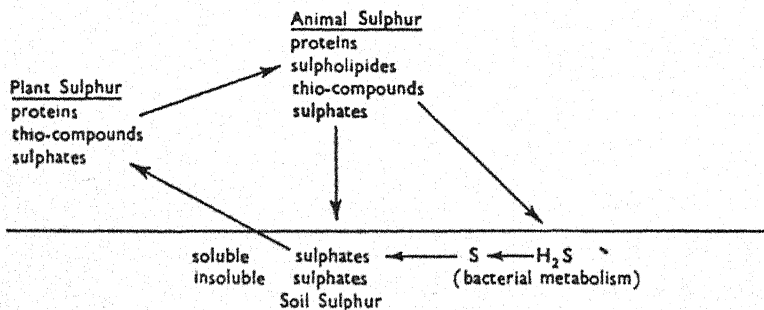
(13) *Thio-melanins.*—Skin pigments containing sulphur and amino-acid residues.

Three principal forms of urinary sulphur are recognised: (i.) sulphate ions, SO_4^{--} ; (ii.) organic sulphate esters of phenols; (iii.) "neutral" sulphur compounds, including thiols and salts of thio-acids.

Significance.—Sulphur assumes so many forms that it is impossible to ascribe a general significance to the element. It is necessary for protein synthesis in plants, although unassimilable as a free element. In the animal dietary, sulphur resembles phosphorus in being acidogenic. It is assimilated in the almost neutral form of thio-amino

acids, obtained from proteins, and is excreted after oxidation as an ion of the inorganic acid, H_2SO_4 . Organic sulphates enter into the detoxication mechanism of the mammal, and are synthesised to enable the organism to eliminate alimentary and other autogenic toxins in a harmless form.

The Sulphur Cycle.—Unlike phosphorus, sulphur appears in both oxidised and reduced forms in the history of life. Completely oxidised as sulphate, it enters the plant from the soil, and is converted into partially reduced organic compounds containing the thiol group $-\text{SH}$ or the disulphide linkage $-\text{S}-\text{S}-$. From these, the completely reduced form, H_2S , is derived by bacterial degradation either in the soil or in the alimentary tract of the animal. Hydrogen sulphide is attacked by the sulphur-oxidising bacteria, with the ultimate formation of sulphate, which is available for plant absorption.



Sulphur Bacteria.—A group of organisms that obtain energy by the oxidation of H_2S into H_2SO_4 , by means of a chain of reactions. Some occur in soil, and may account for the conversion of unavailable sulphur into forms assimilable by higher plants.

The biochemistry of the sulphur bacteria has been reviewed by Bunker (1936).

Group VII : { Manganese.
Halogens : Fluorine, Chlorine, Bromine, Iodine.

Manganese.—Mn, a.n. 25 ; a.w. 54.93. Present in all plants, the values ranging from less than 1 mg. up to about 200 mg. Mn per kg. dry material. Representative figures are : wheat-bran, 100-200 ; beetroot, oats, spinach, wheat-grain, 25-100 ; edible fruits, less than 15. The maximum was found in lettuce, namely, 216.2 mg. Mn per kg. These values vary greatly with soil conditions, and the Mn content of seeds is important, since it may be the

only source available for the growing plant. The Mn content of cereal grain is often equal to or greater than the Fe content. Manganese is widely but unequally distributed as a micro-constituent of higher animals, being most in liver, pancreas, lymph gland, and kidney. Recorded values range from 0.01 mg. Mn per 100 gm. fresh tissue, in muscle, up to 0.2 mg. Mn in liver. Marine animals are richer in the element, and a manganese-protein compound, *pinnaglobulin*, occurs in the blood of the mussel *Pinna squamosa*.

Sea water contains 1-10 mg. Mn per cubic metre.

Significance.—(1) *A Factor in Plant Growth.* Deficiency of manganese causes retardation of growth and chlorosis of the leaf, due to lack of chlorophyll. Slight excess of manganese is toxic, especially to barley.

(2) *An Enzyme Activator.*—Phosphorylase, alkali phosphatase, arginase myosin, dipeptidase, and laccase are activated by manganese.

(3) *A Factor in Bone Development.*—Fresh bone contains about 0.03 to 0.1 mg. Mn per 100 gm. Lack of the element in the diet of chickens leads to *perosis*, a deformity of the tibio-metatarsal joints, and subsequent deformities (Gallup and Norris, 1938).

(4) *A Factor in Growth and Reproduction.*—Manganese acts as a growth-stimulant for young rats, and appears to be necessary for normal reproduction. It is invariably present in the reproductive organs and in the developing egg. Everson and Daniels (1930) claim that it is necessary for child nutrition, and prescribe a daily intake of 0.2-0.3 mg. Mn per kg. body weight during the first five years.

Manganese in Foodstuffs.—From the surveys of Peterson (1928), Richards (1930), the richest sources are: liver, kidney, pancreas, muscle, lettuce, spinach and unmilled cereals. Wheat germ, which contains about 39 mg. Mn per 100 gm. dry material, offers an abundant supply (Sluiter, 1933; Von Oettingen, 1935).

Fluorine.—F, a.n. 9; a.w. 19. Fluorine, the most active of the elements, is a micro-constituent of plants, reported values ranging from 6-1,000 mg. F per kg. fresh tissue.

The fluorine content of sea water is about 1.4 mg. per litre, and the halogen is a frequent micro-constituent of marine organisms.

Higher animals contain fluorine in skeletal tissue and viscera. Values range from 2 mg. in human brain and lung up to 15 mg. in kidney tissue, expressed as mg. F per kg. dry material. Egg yolk contains about 11 mg. per kg. of fluid. Fresh human bone contains 150-560 mg. F per kg., the maximum being in dentine. Tooth substance has been reported to contain 0.2-0.8 per cent. CaF_2 , enamel being specially rich, but Bowes and Murry (1935)

claim that there is not more than 0.03 per cent. of F in both dentine and enamel. These low values may be due to dietary conditions.

Significance.—Fluorine contributes to the hardness of the skeleton, especially the teeth. There are minimal and optimal concentrations in the dietary, beyond which conditions of fluorine deficiency and fluorine poisoning, respectively, are observed. Concentrations of NaF above 0.05 per cent. in the diet of the rat cause pathological over-development of tooth enamel, and defective dentition.

Inorganic fluoride has an inhibitory action on enzymes, especially those causing activation of carbohydrates and fats.

Sharpless and McCollum (1933) find that the fluorine content of bones and teeth varies with the diet, and can be reduced almost to zero without causing a marked change in tooth structure, or affecting the general well-being of the animal, as shown by growth-rate and power of reproduction. Micro-administration of CaF₂ in the form of bone powder, is said to increase resistance to tooth decay. A dental defect has been traced to the use of water rich in fluorides, and mottling of the dental enamel has resulted from the consumption of drinking water containing 3.8–7.15 mg. F per litre.

The physiological effects of fluorine have been reviewed by Dyson (1927), and by McClure (1933).

Chlorine.—Cl, a.n. 17; a.w. 35.46. Chlorine has been detected in all plants, with the outstanding exception of the conifers. It is a constant micro-constituent of seeds. The chloride value of common plants is very variable, ranging from less than 0.005 per cent. of wheat ash up to 9 per cent. of lettuce ash. The halogen is an invariable constituent of the animal body, being greatest in lower marine forms and least in some fresh water species.

Percentages in fresh human tissues and fluids are: blood, 0.28–0.3; plasma, 0.35–0.38; red cells, 0.18; spinal fluid, 0.44; sweat, 0.07–0.52; urine, 0.12–0.06; lung 0.26; muscle, 0.05–0.13; milk, 0.04; gastric juice, 0.1–0.3.

The chloride of the human body amounts to about 0.15 per cent. of the total weight. Concentration is maximal in serum and plasma exudates, gastric juice, spinal fluid, and urine; and is minimal in brain and muscle. Urinary chloride depends on the level in the plasma, and falls almost to zero if chloride be withheld from the diet or if the plasma chloride fall below the limiting value of 0.34 per cent.

Forms of Occurrence.—Tissue chlorides can be extracted by dialysis, and it is concluded that biological chlorine is mostly ionic, although organic forms are known to exist (Nolan, 1936).

Significance.—(1) *A Transport Element.*—Chlorides of the biochemical metals are easily soluble in water and readily ionised. Chlorine thus provides a transport anion for the absorption, distribution, and excretion of other radicles.

(2) *A Factor in Hydration.*—Na and Cl are the chief inorganic ions of the animal, and their concentration is partly responsible for determining the amount of water held by various tissues.

(3) *A Factor in Osmotic Pressure.*—NaCl is the principal electrolyte of the plasma, and largely determines the osmotic pressure level of the tissue fluids.

(4) *A Buffer Agent.*—Cell membranes are more or less permeable to Cl-ions, but relatively impermeable to some metallic ions that accompany Cl. Potassium is located chiefly in cells; sodium is found chiefly in tissue fluids. The Cl-ion is able to migrate between cell and surrounding fluid in response to changes in K^+ or Na^+ concentration. This "chloride shift" occurs in blood when increase in carbonate concentration drives chloride from plasma to red cell, or *vice-versâ*. Conversely, removal of carbon dioxide during pulmonary aeration causes chloride to pass from the red cells to the plasma.

(5) *A Constituent of Gastric Secretion.*—The HCl of gastric juice is essential for peptic digestion.

(6) *A Factor in Plant Growth.*—External chloride does not appear to be necessary for plant growth as the seed carries all the necessary halogen. It is, however, required for seed-production in the mature plant.

Bromine.—Br, a.n. 35; a.w. 79.92. A constant constituent of marine plants and marine animals, especially anthozoa, in which it may replace chlorine almost completely. A dibromo-indigo, the original Tyrian purple of Imperial Rome, is obtainable from the gastropods, *Murex brandaris* and *M. trunculus*. Bromine appears to be a micro-constituent of higher animals, reported values ranging from 0.3 mg. Br per 100 gm. fresh lung tissue up to 1.5 mg. per 100 ml. blood. The occurrence is variable, and nothing is known as to the significance of the halogen.

The Br value of human blood lies between 227 and 572 γ per 100 ml., the average being 372 γ for blood, and 656 γ for urine (Conway and Flood, 1936). Impure table salt, baking powder, and the hypochlorite used in flour bleaching are common sources of bromine in the diet. (1,000 γ = 1 mg.).

Iodine.—I, a.n. 53; a.w. 126.98. A micro-constituent of all plants and animals. Seaweeds are especially rich, and form an industrial source of the element. The kelp ash of *Laminaria digitata* may yield 33–40 lb. per ton. Among land plants, the

values average about 0.0005–0.01 gm. I per 100 gm. fresh material, the concentration depending more on district than on species. Watercress and other fresh-water plants have relatively higher values.

In mammalian tissue, the thyroid is noteworthy for its high content of iodine, values in man range from 9.0–40 mg. I per 100 gm. fresh tissue. Other organs have values ranging from 1 mg. in liver and kidney down to 0.01 mg. in blood. The iodine value of the thyroid depends primarily on the iodine intake, and to a lesser extent on species, age, and sex. The content is usually inversely proportional to the size of the gland, and is subnormal in all conditions of simple goitre. The blood iodine level may rise to 0.04 mg. I per 100 ml. in exophthalmic goitre, and fall to less than 0.003 in simple goitre. The recorded urinary output ranges from 0.03–0.173 mg. *per diem*. Milk contains 0.001–0.046 mg. I per 100 ml.

The adult human body includes 20–50 mg. I.

Forms of Occurrence.—(1) *Thyroxine* occurs as a natural amino acid in the thyroid, and is the active radicle in its internal secretion.

(2) *Iodogorgoic acid*, a di-iodide of tyrosine, occurs in spongin, the skeletal protein of sponges.

Significance.—The influence of iodine on plant metabolism is uncertain. In marine invertebrates, especially sponges and corals, organic iodine forms part of the skeletal protein.

It is an essential micro-constituent of the higher animal, the minimal daily requirement for man being about 0.05 mg. I_2 . Elaborated into thyroxine by the thyroid apparatus, iodine controls the basal metabolic rate of animal metabolism. The distribution of iodine has been surveyed by McClendon (1927), Orr and Leitch (1929), and Orr (1931).

Group VIII : Iron, Cobalt, Nickel.

Iron.—Fe, a.n. 26 ; a.w. 55.84.

Iron is the fourth element in order of abundance in the earth's crust, and is a micro-constituent of all living tissues. Iron is universal in the green parts of plants, the values ranging from about 5–10 mg. per 100 gm. of dry material. It also occurs, but to a much lesser extent, in roots, tubers, and in plants free from chlorophyll. Vegetable foodstuffs in order of decreasing value are : dried legumins, green leafy vegetables, dried fruits, nuts, cereals, green legumins, roots, tubers, fruits.

Iron is universal in animals, its micro-concentration being 0.01–0.1 mg. per gm. of cell substance. In addition, it is an invariable secondary element in all the red-blooded animals. The iron content of mammalian blood is approximately 50 mg. per 100 ml., mostly in the form of *hæmoglobin*, which contains 0.3–0.4 per cent. Fe.

The human adult contains about 0.01 per cent., or 3.5 gm. Fe, in terms of total body weight, of which 2.5 gm. circulates as blood pigment, and 1.5 gm. is stored in spleen, liver, and bone marrow.

Representative iron percentages of fresh animal tissues and fluids are : brain, 0.002 ; hair, 0.08 ; kidney, 0.005 ; liver, 0.008 ; lung, 0.01 ; muscle, 0.004 ; pancreas, 0.006 ; spleen, 0.009 ; human milk, 0.0004 ; cow's milk, 0.0002-0.001 ; hen's egg, 0.0025 ; hen's egg yolk, 0.007.

For a natural food material, milk is remarkably deficient in iron, and the young animal relies largely on the metal stored in its liver before birth.

Iron is absorbed from the intestine as ferrous ions, Fe^{++} , liberated by gastric HCl acting on the food materials. If absorption be delayed, ferrous iron is oxidised to ferric iron in the alkaline regions of the small intestine, or may combine with sulphides and phosphates to form insoluble salts. Under these conditions, iron is non-available, and is excreted by the intestine. Iron in the organic form of hæm is not readily released by gastric digestion. McCance found that 15-18 mg. of iron are retained when 50 mg. are given daily as sulphate or chloride to a subject in a state of iron equilibrium, but none is retained when the 50 mg. are given in the form of hæm. For this reason, muscle and dried blood are poor sources of nutritional iron. Assimilated iron is not returned to the intestine, which, according to McCance, "has no power of regulating by excretion the amount of iron in the body" (1938).

Forms of Occurrence.—Tissue iron occurs as a porphyrin derivative, hæm, from which are derived the cytochromes and other hæmochromes present in all aerobic cells. Iron also occurs as the mobile respiratory pigments of blood, *chlorocruorin* and *hæmoerythrin* in lower animals, and *hæmoglobin* in higher animals. The enzymes *catalase*, *peroxidase*, and *histaminase* are proteins united to hæm.

Significance.—The biological function of iron is the transference of oxygen from the environment to the oxidisable metabolites in the tissues. In higher animals, this is effected by chains of reacting compounds, which include hæmoglobin and cytochrome c.

In the green plant, iron appears as a constituent of the chloroplasts. It is not part of the chlorophyll molecule, but is necessary for chlorophyll formation. When iron is withheld from plants, local chlorosis develops, and can be cured by application of iron salts to the soil.

In the higher animal, iron is stored chiefly in liver and spleen, but only a small part of this is useful for hæmoglobin manufacture. In the treatment of anæmia, the need for large doses of iron depends partly on the low assimilability of the metal, but chiefly on account

of the activity of the storage mechanism, "which must be flooded with iron if any is to overflow and be available for hæmoglobin synthesis."

In some species (human subjects and rodents) copper is an essential supplement of iron in hæmoglobin manufacture (Hutchison, 1938).

A Factor in Plant Growth.—The response of plants to iron depends both on the nature of the salt supplied and the nature of the soil. A slight deficiency of iron can diminish greatly the yield of a crop without causing much change in appearance; an acute deficiency alters the colour of the leaves owing to defective chlorophyll formation.

Oxidation Inhibitors.—Cyanides, hydrogen sulphide, and carbon monoxide react with hæmatin compounds containing ferrous iron, and each of these reagents can inhibit the respiration of all tissues. This led Warburg to conclude that an iron catalyst is concerned in cellular respiration, and his conclusions have been confirmed by the discovery of the cytochromes.

Iron Bacteria.—These higher bacteria inhabit ferruginous waters, and are characterised by a cell membrane impregnated with ferric hydroxide, which results in the formation of fluffy, brownish streamers on stones or plant stems under water. It was at one time believed that these organisms obtained their energy by the oxidation of ferrous salts to ferric, but it is now known that their metabolism is orthodox. The colour is due to local precipitation of iron salts, and is not a unique metabolic process. During their life period, iron bacteria cause the accumulation of ferric hydroxide in natural waters containing soluble iron salts.

The Iron Cycle.—Iron occurs on the surface of the earth chiefly in union with oxygen as ferrous oxide, FeO , and ferric oxide, Fe_2O_3 . The former, FeO , is a strong base, able to form neutral salts with most acids. The latter, Fe_2O_3 , is a weak base, unable to fix CO_2 as a stable carbonate. Ferrous silicates of rocks and soil are decomposed by atmospheric CO_2 , and yield ferrous carbonate. This is oxidised to ferric oxide, and the liberated CO_2 is returned to the atmosphere. Ferric oxide can act as an oxygen donator to the organic matter in the soil, and thus is reduced to ferrous compounds. These are redistributed by the soil water, and reoxidised to ferric oxide. Iron is therefore a continuous oxidising agent, preventing the retention of carbon in soil, and enabling it to return to the atmosphere as CO_2 .

Cobalt.—Co, a.n. 27; a.w. 58-97.

Nickel.—Ni, a.n. 28; a.w. 58-68.

These closely related metals are widely distributed micro-

constituents of plants and animals, reported values ranging from 0.002 to 2 parts per million fresh tissue (Bertrand). In plants, Ni occurs chiefly in the leaf, ranging from 1.51 mg. per kg. dry material in lettuce to 3.3 mg. in cabbage. Both metals were found in many of the marine organisms examined by Fox and Ramage, although neither Co nor Ni has yet been detected in sea water. Bertrand claims that both Co and Ni are normal constituents of the pancreas, and necessary for its functioning. Cobalt has been shown to be effective in raising the blood volume and red cell concentration in rats, and to be a specific cure for the disease, enzootic marasmus, or bush sickness, found in sheep and cattle in some parts of Australia and New Zealand, and now ascribed to deficiency of Co in the soil and herbage (Underwood, 1937).

Values for Co in mg. per kg. are: cacao bean, shelled, 0.03-0.4; coffee bean, 0.04; tea, 0.15; wholemeal flour, 0.01; milk, 0.001; liver and muscle, 0.2; spleen, 0.5.

General Significance of the Biochemical Elements.—When the composition of a higher organism is compared with that of its environment several features of distinction are seen. Thus, silicon and aluminium, that together as the aluminium silicate of clay make up a third of the earth's crust, are found only as micro-constituents in man, and give little indication of a dusty ancestry. On the other hand, hydrogen, carbon, and nitrogen, the building stones of life, occur only as secondary constituents of the environment.

The suitability of the biological elements would appear to be set beyond discussion by the mere fact of their presence in the organism, though there may be a process of chemical selection at work gradually altering and improving the framework of life.

Only eight elements are present in the Earth's crust in concentrations of more than 1 per cent. They are: O, Si, Al, Fe, Ca, Na, K and Mg, and together make up 98 per cent. of the surface. About 1.1 per cent. is made up of Ti, P, Ca and H (as H_2O). The remaining 80 elements together constitute only 0.5 per cent. of the crust.

A 70 kg. human body contains approximately: oxygen, 46 kg.; carbon, 12 kg.; hydrogen, 7.2 kg.; nitrogen, 1.7 kg.; calcium, 1.1 kg.; phosphorus, 630 gm.; chlorine, 112 gm.; fluorine, 60-100 gm.; sulphur, 110 gm.; potassium, 90-100 gm.; sodium, 95 gm.; magnesium, 60 gm.; iron, 4-5 gm. Variations depend on the state of nutrition, the relative fat content and the size of the skeleton.

Approximate percentage values for the three chief micro-constituents of adult man are: Mn, 0.003; Cu, 0.0002; I, 0.0001. It will be seen that the percentage value for the entire organism is not necessarily an index of biological significance, as the element may be

RELATIVE COMPOSITION OF THE HUMAN ORGANISM AND THE ENVIRONMENT

The environment is taken as the crustal layers of the earth, the average composition of which to a depth of 16 kilometres (10 miles) is calculated by analysis of samples from mines and well-borings.

Rough data, such as those tabulated below, show the power of the living organism to select the necessary biological elements irrespective of their relative concentrations, and its inability or reluctance to utilise some of those most readily available.

ORGANISM <i>Percentage composition</i>		ENVIRONMENT <i>Percentage composition</i>	
Oxygen	63.03	46.68	Oxygen
Carbon	20.20	27.60	Silicon
Hydrogen	9.90	8.05	Aluminium
Nitrogen	2.50	5.03	Iron
Calcium	2.45	3.63	Calcium
Phosphorus	1.1	2.72	Sodium
Chlorine	0.16	2.56	Potassium
Fluorine	0.14	2.07	Magnesium
Sulphur	0.14	0.7	Titanium
Potassium	0.11	0.15	Phosphorus
Sodium	0.10	0.15	Carbon
Magnesium	0.07	0.11	Hydrogen
Iron	0.01	0.1	Manganese
Micro-constituents	< 0.01	0.09	Sulphur
		0.09	Chlorine
		< 0.09	Micro-constituents

concentrated in one tissue. For example, the thyroid gland has about 0.06 per cent. of iodine.

The availability of the biological elements fixes the limits to the expansion of life. This was recognised by Liebig in 1843, and expressed as his familiar "law of the minimum."

By the deficiency or absence of *one* necessary constituent, all others being present, the soil is rendered barren for all those crops for the life of which that one constituent is indispensable.

The "minimum" effect, as Lagatu has found for the vine, shows itself as a nutritional unbalance due to increased uptake of the remaining elements (Thomas, 1929).

L. J. Henderson has developed the concept of environmental fitness, and has shown that the unique properties of the primary elements, carbon, hydrogen, and oxygen, endow their compounds with the maximal fitness for the manifestation of life. This concept has not yet been extended to the other elements, although it is interesting to speculate on the biological importance of magnesium and iron. Without the former there would be no chlorophyll, without the latter there would be no hæmoglobin.

Marett (1936) believes that the mineral deficiencies of the soil operate in determining race and destiny.

The Geochemical Changes due to Life.—Life, for all its abundance, does not occupy a large volume of the earth's crust, but the effects of its precarious tenancy are shown by its architectural achievements, the chalk cliffs and coral islands, which are monuments of many thousand years of biological industry. In 1875, Suess introduced the term *biosphere* to denote the portion of the earth occupied by life, and, in 1918, Vernadsky began an investigation into the extent to which the maintenance of this biosphere had influenced the surface history of the earth. The Biogeochemical Laboratory of the Russian Academy of Sciences was created in 1928, and since then important surveys have been published by Vernadsky and by Vinogradov and his colleagues, in support of the conclusion that the chemical composition of an organism is characteristic of the species, and that the composition of living matter in general can be regarded as a function of the atomic weights of the elements, every sixth of which, in periodic order, appears to have a special importance in life.

Geologically ancient types, such as bacteria and foraminifera, are able to concentrate a much wider range of elements than those accumulated by the highest modern types, such as birds and mammals.

In regions uninhabited by life, matter in solution is spontaneously dispersed and distributed, but under the influence of life this dispersion is replaced by aggregation and selection, and solar energy is employed to build up compounds. Ritchie (1939) has pointed out that although the soluble silica in the ocean never exceeds 1.5 parts per million, "our present-day ocean plants have assembled from such a dilution, ten millions of square miles of diatom ooze, and

radiolarian ooze accounts for another two millions of square miles of siliceous accumulations." Similarly, from an atmosphere containing 0.03-0.04 per cent. by volume of CO_2 , has been collected the carbon stored in our vast but spendthrift inheritance of peat, coal, oil-shale and petroleum. Ritchie has calculated that the CO_2 dispersed in 16,125,000 cubic yards of air "is gathered and made potentially efficient by a single tree of some five tons dry weight."

Geology and Health.—There is a direct relationship between rock, subsoil, soil, herbage, and the health of grazing animals and man. This is shown by the occurrence of diseases due to lack of essential micro-nutrients normally provided by the soil. These regional deficiency diseases have been studied, since 1935, by Marston and his colleagues, who have shown that daily administration of 1 mg. of cobalt can cure grazing animals suffering from "wasting disease" (W. Australia), "bush sickness" (New Zealand), "moor cling" (Devon), and "pining" (Scotland); while iron and copper administration has cured "salt-sickness" (Florida). Similar types of cure have been reported by Dunlop and McCallien (1941).

A converse pathological condition can occur when toxic elements, such as Be, As and Se, accumulate in plants grown on certain soils. This is not always due to excess of the element in the soil, but may arise because a particular species of plant has a special affinity for the element. Some leguminosæ, notably those of the genus *Astragalus*, by their preferential growth will reveal the presence of selenium in soil from areas capable of producing forage or grain that is poisonous owing to its high content of Se. These leguminosæ are now used in soil surveying, as *Indicator plants* (Trelease, 1942).

Tracer Isotopes in Biological Research.—Isotopes of a chemical element are atoms of the same atomic number and chemical species but of different atomic weight. During the early history of the Earth the isotopic forms of the various elements have become mixed so uniformly that the proportion of isotopes in any ordinary sample of an element is approximately constant, and the element consequently has an "average atomic weight" that is not necessarily a whole number. Isotopes differ in atomic weight by one or more units, owing to the presence of one or more extra neutrons in the atomic nucleus. The chemical properties remain the same, as they reside in the outermost shell of electrons, which is the same for all atoms of the same chemical species.

Whenever hydrogen occurs in Nature, the proportion of heavy hydrogen, *deuterium*, ^2H , or D, is uniformly about 0.02 per cent., and is independent of the source, which may be sea water, lake water, or biological liquids. The same proportion is also found in organic compounds, which ultimately arise from CO_2 and H_2O ,

showing that the living cell does not sharply discriminate between either form of hydrogen.

About 0.37 per cent. of the atoms of nitrogen in natural organic and inorganic compounds occur as the heavy isotope ^{15}N .

Sources of Isotopes.—Heavy isotopes can be fractionated from naturally occurring mixtures by mechanical methods involving electrolysis and distillation, such as have been developed by Urey and his collaborators. Radioactive and other isotopes can also be prepared artificially by bombarding elements by high-speed nuclear particles, such as protons (H^+), deuterons (D^+) and neutrons; now obtainable in quantities by means of the *cyclotron*, invented by E. O. Lawrence, in 1932. Isotopes are estimated by means of the *mass-spectrograph* (Aston, 1920), which can measure individual atomic weights, with an error of less than 1 in 10,000. Radioactive isotopes can be measured by means of the Geiger electron-counter.

Application to Biology.—Compounds labelled by replacing part or all of a particular element by its identifiable isotope are of unique value in tracing pathways of metabolism in plants and animals. In 1926, von Hevesy introduced this use of isotopes as tracers. By adding a radioactive isotope of lead to the diet, he was able to follow the Pb distribution in the animal body from the radioactivity of the ash obtained from different organs, and, in 1937, by means of the isotope ^{32}P , he showed that phosphorus in plants and animals is a very labile element, and, even in structural tissues such as bone and teeth, is being continually exchanged with circulating phosphate. In the meanwhile, Schoenheimer, Rittenberg and their colleagues began their important study of the fate of organic compounds labelled by inclusion of D or ^{15}N , which has revealed much of the metabolic history of proteins, carbohydrates and lipides.

D is obtained from "heavy-water" fractions rich in DHO and D_2O , some of the deuterium of which ionises as D^+ , and exchanges spontaneously with the labile H in NH_3 and in reactive groups such as $-\text{OH}$, $-\text{CHO}$, $-\text{COOH}$ and $-\text{NH}_2$. Hence, organic compounds labelled by inclusion of D must have the isotope inserted in a stable group, such as a terminal $-\text{CH}_3$, or an intermediary $-\text{CH}_2-$ or $-\text{CH}=-$; otherwise, the labelled compound will lose some of its isotope by exchange with H_2O within the organism.

Heavy carbon, ^{13}C , and artificially prepared radio-active carbon, ^{14}C , supplied in the form of carbon dioxide, are used to trace carbon uptake and transport. Heavy nitrogen, ^{15}N , is used to trace nitrogen uptake by plants, and nitrogen transport in animals. Other isotopes employed in elucidating special lines of metabolism include radio-sulphur, ^{35}S , radio-iron and radio-iodine. Where an element has several radio-active isotopes, the one selected has a "half-lifetime"

sufficiently long to enable it to persist in the animal body during the duration of the experiment.

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CHAPTER 3

INORGANIC COMPOUNDS

"One may not doubt that, somehow, Good
Shall come of Water and of Mud ;
And, sure, the reverent eye must see
A Purpose in Liquidity."

RUPERT BROOKE.

WITH the exception of oxygen, and traces of dissolved hydrogen and nitrogen, elements do not occur free in living organisms, but are present as inorganic and organic compounds. As a class, inorganic compounds are non-combustible, and, with the exception of the carbonates, do not contain carbon. Bio-organic compounds, on the other hand, are combustible, and all contain carbon and hydrogen.

Animal or vegetable ash after complete incineration is composed entirely of inorganic salts and oxides, and such compounds were the first investigated. Subsequently, analyses were made of tissue extracts and secretions after separation of organic compounds by dialysis or precipitation. This method gives more significant information, since it deals with salts and ions actually present during life, but, even so, there is no doubt that many inorganic radicles occur united to organic compounds, both in hard tissues, such as bone, and in soft tissues, such as muscle.

Inorganic Biochemical Compounds.—The most important are : water, carbon dioxide, the carbonates, carbamates, silicates, sulphates, phosphates, fluorides, and chlorides of the biochemical metals ; the nitrogen derivatives, ammonia, nitrous and nitric acid.

Water.—Water comes first in quantitative importance among biochemical compounds, and is the solvent in all vital reactions. The average water content of land animals is about 60–70 per cent. of the total weight. It may be as low as 10 per cent. in some insects, and in latent forms of life, such as seeds and spores ; and may exceed 95 per cent. in lower forms of marine life, such as jelly fish.

The water content of mammalian tissue is roughly proportional to physiological activity, and inversely proportional to fat-content. It decreases with age. The human embryo at the end of the third month contains about 94 per cent. At birth, this has fallen to 67, and in adult life it is fairly constant between 60–63 per cent.

The 40–45 kg. of water incorporated in the human adult are located chiefly in the muscles. Adolph (1933) computes the total

turnover of water between tissues, blood and alimentary tract, to range from 4.75 to 17.6 litres *per diem*, involving a loss of 1.05-7.8

Percentage Water Content of Human Tissues

Tissue.	New-born.	Adult.
Blood . .	85.0	77.9-83.0
Muscle . .	81.8	73.0-75.7
Brain . .	89.3	75.0-82.0
Bone . .	32.3	22.0-34.0
Lung . .	82.6	78.0-79.0
Liver . .	80.5	68.3-79.8
Kidney . .	85.7	77.0-83.7

litres, through the channels of the kidneys, colon, lungs and skin, the average daily output being 3.4 litres. This must be made good by the water obtained from the diet.

Estimation of Water.—This may be done by drying the tissue or other material to constant weigh (1) in a steam oven at 96° C., or (2) in a hot-air oven at 110-115°, or (3) in a vacuum oven at 70° and less than 50 mm. Hg pressure; or, more rapidly, by adding an excess of toluene (B.P. 110°), or other non-miscible liquid, and then distilling, when the toluene carries over the water, which separates out in a lower layer, and can be measured directly in a graduated receiver. Different methods may yield different results for the same material, and the technique adopted should always be specified.

Free Water and Bound Water.—The organism is a cellular, colloidal structure, and contains water in two chief forms: free water, or *water of solution*; and bound water, or *water of hydration*. Water in the liquid phase consists of units of 5 molecules, the central one being surrounded by four others tetrahedrally placed. Water of hydration consists of water molecules bound to *hydrophil* acceptors: proteins and other tissue colloids, soaps, and some inorganic ions, notably Na^+ .

Living cytoplasm, according to Martin Fischer, is a hydrophil colloidal aggregation, and the various solutes of cytoplasm are held dispersed in the colloidal hydrates. Free water circulates as an excretory solvent, and varies according to diet, metabolism, and activity. Bound water is an integral part of the tissues, and reaches a constant value in mature life.

Functions of Water.—(1) *Structure.*—Water determines the bulk of tissues and organs, and renders them plastic while incompressible.

(2) *Nutrition*.—All the food materials of plants and animals are assimilated in aqueous solution. Water, in addition, is the chief quantitative constituent of the dietary.

(3) *Anabolism*.—In green plants, water is utilised in the synthesis of bio-organic compounds, including carbohydrates.

(4) *Catabolism*.—Water is the major end-product of plant and animal metabolism.

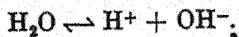
(5) *Transport*.—The food materials, the internal secretions, and the waste products of the organism are distributed in aqueous solution.

(6) *Temperature Stabilisation*.—The high specific heat of water enables the organism to store heat and maintain a uniform temperature. The high latent heat of water renders it very efficient as a cooling agent when evaporating from the surface of the organism.

(7) *Solvent*.—Water is a powerful ionising solvent, and ionisation is a preliminary stage in almost all the reactions taking place within the organism.

Ionic Systems.—All aqueous solutions can be divided into (a) electrolytes, and (b) non-electrolytes. Electrolytes are characterised by their ability to conduct electricity, and by the fact that many of their physical properties, such as osmotic pressure and boiling point, are in excess of those calculated from the molecular weight of the solute, showing that solution has been attended by an increase in the number of particles dissolved. Non-electrolytes have minimal electric conductivity, and their properties indicate that no increase has taken place in the units of solute. The difference is ascribed to the presence of *ions* in the electrolytes. These are derived spontaneously from the solutes, and their concentration determines the conductivity of the solution. Solutions containing no ions have zero conductivity.

Water is the most effective of the ionising solvents, and even when pure has a low conductivity of its own, indicating a slight degree of ionisation :



The H^+ , or proton, being highly reactive, unites with a water molecule to form the *hydroxonium* ion, H_3O^+ , which is also written $\text{H}_2\text{O} : \text{H}^+$, to show that a proton is in semi-polar union with H_2O , and can be removed easily by a proton acceptor.

Salts, even of weak acids or bases, are almost completely ionised in aqueous solution, consequently all the tissues and fluids of the organism contain a mixture of various ions. Some of these mixtures form natural systems of considerable importance in life. These include :

(1) *The hydrogen ion buffer system*, whereby the acidity and alkalinity of the tissues are kept within proper working range.

(2) *The oxidation-reduction systems* participating in tissue metabolism.

(3) Various *metallo-ionic systems* influencing tissue water content, and physiological sensitivity.

The H-ion Concentration of Living Tissues.—Since all forms of life contain water, and all aqueous solutions contain H-ions and OH-ions, it follows that these are invariable constituents of living tissues. In pure water, or in other neutral solutions, the H-ion concentration, usually written $[H^+]$, exactly equals the OH-ion concentration, or $[OH^-]$. In acid solutions, $[H^+]$ is greater than $[OH^-]$. In alkaline solutions, $[OH^-]$ is greater than $[H^+]$. However they may vary individually, the product $[H^+] \times [OH^-]$ has a constant value for a given temperature. That is to say, neither $[H^+]$ nor $[OH^-]$ can ever equal zero. The strongest acid solution obtainable contains OH-ions; and the strongest alkaline solution contains H-ions, present as H_3O^+ .

The activities of life are essentially acidogenic in that carbon dioxide is an end-product of the oxidation of all organic compounds. In consequence, the H-ion concentration of the tissues and tissue fluids is continually tending to increase, and must be neutralised by appropriate bases if the chemical equilibrium of the organism is to be preserved.

Acids are substances capable of liberating H-ions.

Bases are substances capable of combining with H-ions.

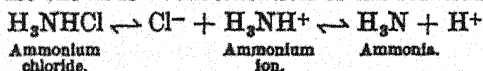
Acid.	Ions Liberated.	Percentage Ionisation in 0.1 M Solution.	Dissociation Constant (K) $= \frac{[H^+] \times [A]}{[HA]}$
Hydrochloric	$H^+ + Cl^-$	>90	> 10^8
Sulphuric	$H^+ + HSO_4^-$	>90	> 10^8
Sulphurous	$H^+ + HSO_3^-$	34	1.7×10^{-2}
Phosphoric	$H^+ + H_2PO_4^-$	24	7.6×10^{-3}
Acetic	$H^+ + CH_3COO^-$	1.33	1.8×10^{-5}
Carbonic	$H^+ + HCO_3^-$	0.18	3.1×10^{-7}
Hydrogen sulphide	$H^+ + HS^-$	0.09	8×10^{-8}
Boric	$H^+ + H_2BO_3^-$	0.01	6×10^{-10}

It will be seen from this table that dihydro- and trihydro-acids only release one of their acidic hydrogens in 0.1 M concentrations. By greatly increasing the dilution of the acid, all the acidic hydrogen is ionised, whatever be the nature of the acid. Acid salts, such as NaH_2PO_4 , $NaHSO_4$ and $NaHCO_3$, also liberate H-ions, but to a much lesser degree than the parent acid. The ionisation of very strong acids, such as HCl, can only be computed approximately in concentrated solutions, owing to ionic attraction effects.

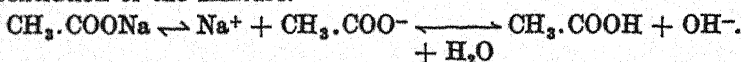
In the anhydrous state, acids, such as HCl gas, pure HNO_3 and pure H_2SO_4 , contain no free H-ions. On addition of water, or other ionising solvents, ionisation takes place to an extent determined by the *strength* of the acid. Strong acids are almost completely ionised; weak acids, except in great dilution, are only partially ionised. The degree of ionisation is expressed either as (i.) the percentage of acid present in the form of free ions, or as (ii.) the *dissociation constant* of the acid, K , which is the product of the concentrations of both component ions, $[\text{H}] \times [\text{A}]$, divided by the concentration of the unionised molecules of acid, $[\text{HA}]$.

Bases undergo a corresponding type of ionisation in aqueous solution, and liberate OH-ions, which are able to combine with H-ions to form water. The strength of a base is due to the number of OH-ions liberated at a given dilution. Thus, NaOH and KOH are almost completely ionised in N/10 concentrations, while N/10 NH_4OH , a weak base, is ionised only to the extent of 1.9 per cent.

Salts are compounds formed by the union of acidic and basic radicles. When dissolved in water they are ionised into their constituent positive and negative ions. If the positive ion is derived from a weak base, such as ammonia, it tends to lose a proton, and so increase the H-ion concentration of the solution.



Conversely, the salt of a weak acid and a strong base forms an alkaline solution, owing to the tendency of the weak anion to abstract H-ions from the water, and so increase the OH-ion concentration of the mixture.



Neutral solutions are those in which the H-ion concentration exactly equals the OH-ion concentration. Pure water is taken as the absolute standard of neutrality. At a temperature of 22°C ., a litre of water has 10^{-7} *equivalents* of H^+ and 10^{-7} *equivalents* of OH^- . Otherwise expressed, there is 1 gm. of H^+ and 17 gm. OH^- in 10^7 , or 10,000,000, litres of water at 22°C .

For brevity, these concentrations in gram-ions per litre are written C_H and C_OH . The C_H of pure water, or any neutral solution at 22°C . is 10^{-7} . Any solution with a C_H greater than 10^{-7} is acid; and any solution with a C_H less than this value is alkaline. The product $\text{C}_\text{H} \times \text{C}_\text{OH}$ always equals 10^{-14} , so either acidity or alkalinity can be expressed in terms of C_H , which is written $[\text{H}^+]$.

$$[\text{H}^+] \times [\text{OH}^-] = k \text{ (a constant),}$$

$$\therefore [\text{H}^+] = \frac{k}{[\text{OH}^-]}, \text{ and } [\text{OH}^-] = \frac{k}{[\text{H}^+]}$$

Electrometric Determination of C_H . The reaction, or H-ion value of a solution can be measured by three different types of instrument: (i.) the *hydrogen electrode*, (ii.) the *quinhydrone electrode*, and (iii.) the "glass" electrode. In the first method, a platinum electrode with a layer of adsorbed hydrogen is immersed in a solution of known C_H , a similar electrode being immersed in the solution of unknown C_H . The two solutions are connected by a narrow tube containing an electrolyte, KCl, kept from diffusing by means of gelatin or agar (Fig. 1).

H-ions tend to leave each electrode and pass into solution, the extent of their migration being opposed by the concentration of the H-ions already present in each solution. If the two solutions have the same C_H , the same number of H-ions will leave each electrode, and if the electrodes are connected with a suitable potentiometer, no difference in electropotential can be found to exist between them.

If, however, one solution has a lower C_H than the other, that solution will allow more H-ions to migrate from its electrode, which in consequence will become negatively charged when compared with the other electrode.

Knowing the potential difference, e , between the electrodes, and the value of C_H for one solution, the H-ion concentration in the other solution can be found from the formula, in which C_H^1 is the H-ion concentration in the more acid solution:

$$e = 0.0577 (\log_{10} C_H^1 - \log_{10} C_H^2) \text{ volts.}$$

It is now customary to employ the symbol pH instead of the more cumbersome $-\log_{10} C_H$, when the formula becomes

$$-e = 0.0577 (pH^1 - pH^2), \text{ or } pH^1 = pH^2 - \frac{e}{0.0577}.$$

In the quinhydrone method, the platinum electrodes are not kept coated with hydrogen, but, instead, a little quinhydrone ($C_6H_4O_2 \cdot C_6H_6O_2$) is added to each solution. Quinhydrone is a compound, the oxidation-reduction state of which depends on the C_H of its solvent, and this in turn affects the potential of the immersed electrode. The readings and calculation are similar to those of the hydrogen electrode method, and the procedure is more convenient, but has the serious limitation of being inapplicable to alkaline solutions, which destroy the quinhydrone.

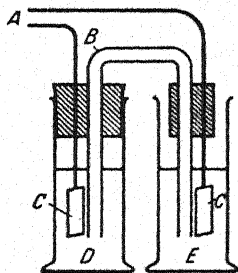


FIG. 1.—Cell composed of two quinhydrone electrodes. A—leads to apparatus for measuring E.M.F. B—siphon filled with KCl in agar. C—platinum electrodes. D—solution of known p_H . E—solution of unknown p_H .

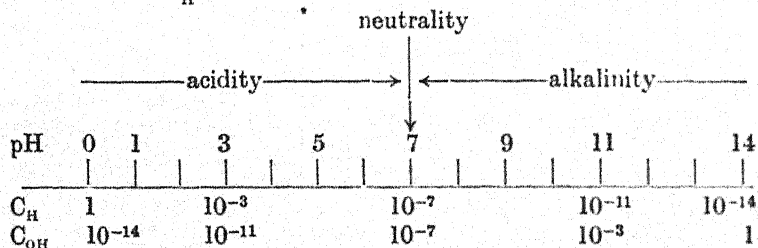
In the "glass" electrode method, the two solutions are separated by means of a thin glass membrane, and the potential difference of the electrodes is measured by means of a specially sensitive potentiometer.

The pH Notation.—The reaction of any solution or tissue can be expressed as 1×10^{-n} gm. H^+ per litre. In practice, this is abbreviated, and written in terms of the exponent n , which is called the pH of the solution. Otherwise defined, pH is equal to the logarithm of the volume in litres of solution that contains 1 gm. H^+ , or, more briefly,

$$pH = -\log_{10} C_H, \text{ and } C_H = 10^{-pH}.$$

True neutrality at $22^\circ C$. is pH 7, and 7 is the logarithm of 10,000,000, the volume of water in litres that contains 1 gm. H^+ . The more acid the solution the smaller will be the volume containing 1 gm. H^+ , and the lower the pH. Hence, *all acidic solutions have a pH value below 7, and all alkaline solutions have a pH value above 7.*

To appreciate the pH notation it is important to remember that pH *decreases* with *increase* in acidity, and, furthermore, since pH is logarithmic, each change in a pH unit represents a ten-fold increase or decrease in C_H .



The pH Scale.— H -ion concentration, or *actual acidity*, of a solution must be distinguished clearly from *available* or *titration acidity*, which is measured by finding the amount of alkali required to neutralise the solution. The pH value depends on the quality as well as the quantity of the acid; the titration value depends only on the quantity of total available acid. That is to say, pH is a measure of the number of acidic ions, while titration acidity is a measure of the number of acid molecules present, irrespective of whether they are ionised or not.

Decinormal HCl is ionised to the extent of about 90 per cent., and has a pH of about 1.04; decinormal acetic acid is only ionised to the extent of about 1.3 per cent. and has a pH of about 2.85.

Similarly, N/1 HCl (3.65 per cent., $pH = 0.09$) is a corrosive liquid, while N/1 $CH_3.COOH$ (6.0 per cent., $pH = 2.37$) resembles the weak vinegar that it so often impersonates.

Equal volumes of decinormal hydrochloric and acetic acid have the same neutralising power for alkalis, although one is a very strong acid and the other is a very weak acid.

Biologically, the actual reaction or pH of a solution is more significant than the neutralising power in terms of a standard acid or alkali.

Actual Acidity

Depends only on the concentration of free H-ions present.

Estimated, electrometrically or colorimetrically.

Expressed in terms of $[H^+]$ or of pH.

Available Acidity

Depends on the number of acid molecules present, irrespective of ionisation.

Estimated by titration with standard alkali.

Expressed in terms of alkali required to neutralise a given volume.

Neutralisation Curves.—When a strong acid is titrated with a strong base, and the accompanying changes in pH are measured, a characteristic curve is obtained showing an enormous alteration in pH near the neutral point. A single drop (0.05 ml.) of decinormal acid or alkali is sufficient to shift the pH value down or up by about six units. When a weak acid is titrated with a weak base, a different type of curve is obtained, in which the equilibrium point is reached gradually, and there are no sudden changes in pH.

Buffer Systems.—Solutions of weak acids and their salts, or weak bases and their salts, constitute buffer systems, so called because they are able to neutralise alkalis or acids without undergoing marked change in pH. For example, sodium acetate can depress the acidity of HCl, and is used for this purpose in the phenylhydrazine test for sugars (p. 128).

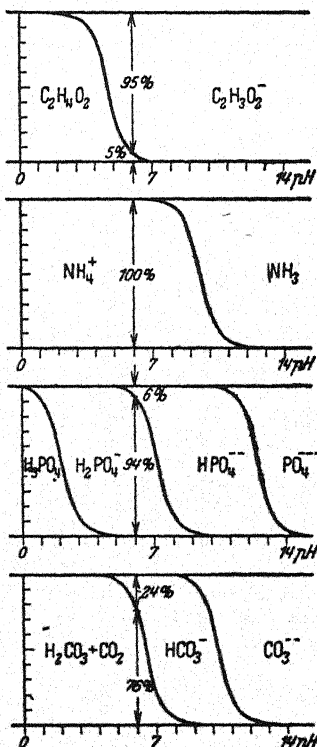


FIG. 2.—State of acids and bases at different pH values for acetic acid, ammonia, phosphoric acid and carbonic acid.

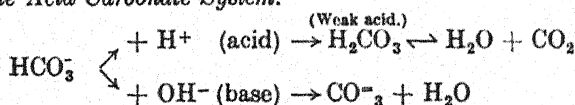
The H-ion concentration of a typical buffer system can be found from the equation,

$$C_H = K \frac{[\text{free acid}]}{[\text{anion}]}$$

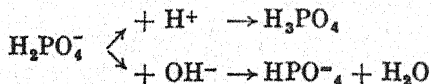
where K is the dissociation constant of the acid.

In a half-neutralised solution, the concentrations of free acid and anion are equal, and C_H becomes equal to K. Hence the dissociation constant of an acid (or a base) can be found by determining the C_H or pH of a half-neutralised solution. The pH of such a solution is termed the pK, or dissociation exponent of the acid (or the base, when an alkali is half-neutralised). Furthermore, the C_H of a half-neutralised solution is almost independent of dilution, since it is determined by the dissociation constant which, unlike the degree of dissociation, does not alter with dilution. Thus, a solution containing equivalent amounts of acetic acid and acetate ions has a pH of 4.75, and a solution containing equivalent amounts of ammonium ions and ammonia molecules has a pH of 9.48, (Fig. 2).

(1) *The Acid Carbonate System.*



(2) *The Acid Phosphate System.*



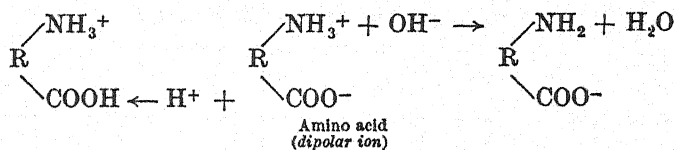
(3) *The Protein System.*

Of these systems, protein buffers are probably of greatest importance in tissues and the blood; the carbonates come next and constitute the bulk of the alkaline reserve of the animal. Phosphate and phosphagen systems operate during muscular contraction. In blood plasma the normal ratio of $\text{HCO}_3^- : \text{H}_2\text{CO}_3$ is 20 : 1.

At the reaction of blood, pH 7.4, this acid carbonate system is not exerting its maximum effect, but as the acidity of the blood increases the buffer action becomes more powerful, and thus constitutes an important alkaline reserve of the organism. The acid phosphate system is more effective at pH 7.4, but the low concentration of phosphates in blood renders the system almost insignificant.

Proteins and amino acids, the structural units of the protein molecule, owe their buffering power to the fact that they exist in solution as dipolar or *zwitter*-ions, each of which carries positively charged $-\text{NH}_3^+$ groups and negatively charged $-\text{COO}^-$ groups. The modern concept of acidity and alkalinity, largely due to the

work of the Danish school of physical chemistry, has shown that the $-\text{NH}_3^+$ group acts as an acid by releasing its proton to form H_2O by union with the OH^- ion provided by a base; while the $-\text{COO}^-$ group acts as a base by combining with a proton, so as to form the un-ionised carboxyl group $-\text{COOH}$.



The modern definition makes it possible to arrange all acids and bases in one table in order of their dissociation, and their strength can be expressed either in pH units or in terms of $[\text{H}^+]$ or of $[\text{OH}^-]$.

Calculation of pH from K.—By the law of mass action,

$$K[\text{AC}] = [\text{A}^-] \times [\text{C}^+].$$

The symbols in brackets denote *concentrations in mols per litre*, where a *mol* is the formula-weight in grams (gram-molecules or gram-ions). $[\text{AC}]$ is the undissociated salt or acid, and the derived anions and cations are denoted by $[\text{A}^-]$ and by $[\text{C}^+]$, respectively.

For exact work, it is necessary to express concentration in terms of *activity*, which is got by multiplying each concentration by a special factor, the *activity constant*, which depends on the size and electric properties of the particles and the nature of the solvent.

K , the *ionisation constant*, *dissociation constant*, or, for acids and bases, the *affinity constant*, has a definite value for each electrolyte for a given temperature. When it refers to an acid or a base it is usually written K_a (acid dissociation constant) or K_b (base dissociation constant). Acids with more than one ionisable H have more than one K_a , marked by a suffix to denote the ionisation of a second or third H^+ .

From the table on p. 53 it will be seen that the K_a values for the common biological acids are very small numbers and, for simplicity in classification, it is now usual to express K_a and K_b in terms of their negative logarithms, $\text{p}K_a$ and $\text{p}K_b$, as is done in the pH notation for expressing $[\text{H}^+]$ and $[\text{OH}^-]$. Here, also, the lower the value of $\text{p}K$ the greater the value of K and the stronger the acid or the base.

$$K = 10^{-\text{p}K}; [\text{H}^+] = 10^{-\text{pH}}.$$

Representative $\text{p}K_a$ values for biological acids at ordinary temperature are: formic, 3.75; lactic, 3.8; benzoic, 4.2; acetic, 4.75; uric, 5.8. Values for acids with more than one acidic hydrogen are: oxalic ($\text{p}K^1$ 1.23, $\text{p}K^2$ 4.19; phosphoric, ($\text{p}K^1$ 2.12, $\text{p}K^2$ 7.2, $\text{p}K^3$ 12.3).

Values of $\text{p}K_b$ for common bases are: ammonium hydroxide, 4.74; methylamine, 3.36; calcium hydroxide, ($\text{p}K^2$) 1.51.

No acid, or base, however strong, is completely ionised in concentrated solution. The effects of dilution in promoting ionisation can be shown by comparing the observed values for a strong acid, such as HCl ($K > 10$), and a weak acid, CH_3COOH ($K = 1.8 \times 10^{-5}$), with values calculated for an imaginary acid ($K = \infty$), which is supposed to be completely ionised at all dilutions.

Concentration	Imaginary Acid		HCl		CH_3COOH	
	$[\text{H}^+]$	pH	$[\text{H}^+]$	pH	$[\text{H}^+]$	pH
10 N . . .	1×10^1	-1.0	?	?	?	?
N . . .	1×10^0	0.0	8×10^{-1}	0.1	4.3×10^{-3}	2.37
N/10 . . .	1×10^{-1}	1.0	9.1×10^{-2}	1.04	1.3×10^{-3}	2.88
N/100 . . .	1×10^{-2}	2.0	9.5×10^{-3}	2.02	4.3×10^{-4}	3.37
N/1,000 . . .	1×10^{-3}	3.0	9.7×10^{-4}	3.01	1.6×10^{-4}	3.87
N/10,000 . . .	1×10^{-4}	4.0	9.8×10^{-5}	4.01	4.3×10^{-5}	4.37

This table shows that a change in pH by one unit represents a ten-fold increase or decrease in $[\text{H}^+]$. Although $[\text{H}^+]$ in aqueous solutions can never equal zero, since the product $[\text{H}^+] \times [\text{OH}^-]$ is a constant and has a real value, it is possible for pH to be 0.0, or even -1, if the solution is sufficiently rich in H^+ .

The strength of an acid is determined by the bond holding the proton, H^+ , to the base. The hydrogen atom contains one proton and one electron; if the electron be lost, the residual proton, H^+ , has a very small volume, and can pack more closely and be held more firmly than larger ions, such as Na^+ . Hence, CH_3COONa ionises freely as CH_3COO^- and Na^+ , while CH_3COOH has its H^+ firmly attached, ionises feebly, and is a weak acid. In dilute acetic acid, the acetate anions and the water molecules compete for the H^+ , but since the acetate is more strongly basic, even in N/100 dilution over 90 per cent. of the protons remain attached to the acetate ion when equilibrium is reached.



Calculation of pH from $[\text{H}^+]$.—Given that $[\text{H}^+]$ for N/10 HCl is 9.1×10^{-2} , find the pH.

$$\begin{aligned} 10^{-\text{pH}} &= [\text{H}^+] = 9.1 \times 10^{-2} \text{ for N/10 HCl,} \\ \text{pH N/10 HCl} &= -\log_{10} [\text{H}^+] = -(\log_{10} 9.1 + \log_{10} 10^{-2}) \\ &= -(0.959 - 2) = 1.041. \end{aligned}$$

Calculation of $[\text{H}^+]$ from pH.—Since pH is the negative logarithm of a number, and the logarithm tables are of positive numbers, the pH value must be subtracted from the next highest integer, so as to give two terms, a negative integer and a positive fraction.

Given that the pH of N/10 CH_3COOH is 2.886, find the $[\text{H}^+]$.

$$\begin{aligned} \text{pH} &= 2.886 = -3 + 0.114. \\ [\text{H}^+] &= 10^{-\text{pH}} = 10^{-2.886} = 10^{-3} \times 10^{0.114} = 10^{-3} \times 1.3, \\ \text{since } 0.114 &= \log_{10} 1.3. \end{aligned}$$

Hence, the $[H^+]$ of N/10 acetic acid is 1.3×10^{-3} .

Calculation of pH of Buffer Systems.—The mass action equation, $[H^+] = K_a[HA]/[A^-]$ may be written

$$\log_{10} [H^+] = \log_{10} K_a + \log_{10} \frac{[HA]}{[A^-]}, \text{ or } -\text{pH} = -\text{p}K_a + \log_{10} \frac{[HA]}{[A^-]}.$$

Rearranged, this becomes the *Henderson-Hasselbalch equation*,

$$\text{pH} = \text{p}K_a + \log_{10} \frac{[A^-]}{[HA]}.$$

For a buffer solution containing a weak acid and its ions, K_a is almost constant within a wide dilution range, and can be measured. Hence, knowing the components of a buffer mixture, the pH can be found, or, conversely, knowing the pH, the composition can be calculated.

Maximum buffer effect occurs when $[A^-] = [HA]$, that is, when the acid is half neutralised.

Here, $[A^-]/[HA] = 1$, and $\log_{10} \frac{[A^-]}{[HA]} = 0$, and, by substitution in the equation, $\text{pH} = \text{p}K_a$.

H-ion Indicators.—These are reagents that change colour sharply at particular changes in H-ion concentration. A series has been prepared covering the entire range from extreme acidity, pH 0.1, to extreme alkalinity, pH 13.5 [cf. Appendix].

The H-ion Concentration of the Environment.—(1) *The Ocean.*—Sea water is slightly alkaline, the usual range being pH 7.75–pH 8.25. It is increased by the photosynthetic activity of marine organisms, and Atkins has recorded a value as high as pH 9.7 in the waters of Plymouth Sound.

(2) *The Soil.* Fertile soil has a range of about pH 3–pH 10. Values as acid as pH 1.7 have been found in America, and as alkaline as pH 11 in Egypt. Chalk soil can never be more alkaline than pH 8.4.

The pH of soil is of primary importance in determining the growth and distribution of plants. Sugar beet, for example, grows best between pH 8 and pH 6; flax grows best between pH 6 and pH 4.

The H-ion Concentration of the Organism.—As a rule, plant tissues and fluids are acid, the average value being about pH 5.2; whereas animal tissues at rest, and tissue fluids, tend to be stabilised at a slight degree of alkalinity, about pH 7–pH 7.5. Metabolism is generally acidogenic in character owing to the liberation directly or indirectly of carbonic acid. Gastric juice is remarkable in having an acidity nearly as great as N/10 hydrochloric acid.

Average pH Values of Human Tissue Fluids and Secretions

Blood . . .	7.35-7.5	Gastric juice . .	1.7-2.0
Urine . . .	5.0-7.0	Pancreatic juice .	7.8
Milk . . .	6.5-7.0	Intestinal juice .	7.7

In man, the range of the tissue fluids, or "internal environment," is stabilised within the limits pH 7.0 and pH 7.8. The acid limit is marked by the onset of acidosis and coma; the alkaline limit is marked by the onset of tetany.

Control of H-ion Concentration in Man.—Four main factors operate :—

- (1) The buffer systems of fluids and tissues.
- (2) The respiratory excretion of CO_2 by the lungs.
- (3) The compensatory excretion of acids or bases by the kidney.
- (4) The metabolic formation of ammonia in the kidney and, probably, elsewhere.

Carbon dioxide and carbonic acid.—Oxidised carbon circulates in five forms within the organism: the anhydride CO_2 , as a gas and as a solute; carbonic acid $(\text{HO})_2\text{CO}$; acid carbonate, HO.C(O)O^- ; carbamate, HO.C(NH)O^- ; and is immobilised in the skeleton as normal carbonates, MCO_3 , where M is a divalent metal.

Gaseous carbon dioxide is the form in which the element carbon enters the plant and escapes from the animal. Within the organism it is dissolved in the tissue fluids, and hydrated to form carbonic acid, which in turn is neutralised by the buffer systems.

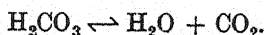
Under ordinary conditions of life, an adult man of 60-70 kg. excretes 750-900 gm. of CO_2 *per diem*. This corresponds to a level of 45-56 ml. CO_2 per 100 ml. arterial blood, and a CO_2 pressure in the alveolar air of the lungs of 40 mm. Hg.

Carbonic acid is the principal "volatile" acid produced in metabolism. It is sufficiently strong to form stable salts with the metals, and yet sufficiently weak to constitute one of the buffer systems whereby the neutrality of the organism is maintained.

The reaction of the blood is being thrust continuously towards the acid side by the CO_2 liberated during metabolism, and this increase in acidity, in turn, serves to stimulate the respiratory mechanism whereby CO_2 is eliminated. Hence, this waste product is utilised as long as it is in the organism.

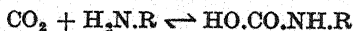
Carbon dioxide transport by the blood.—If pure water is exposed to carbon dioxide at a partial pressure of 40 mm. Hg, which is the usual alveolar value, the gas dissolves until the resulting solution

of carbonic acid has a pH of 4.7 for ordinary temperature. If, however, blood be exposed to the same partial pressure of gas the resulting solution has a pH of about 7.4, which is only slightly less than the value for untreated blood. That is to say, blood has a considerable buffering power for H-ions. But blood is the transport medium for the elimination of carbon dioxide by the lungs, and must be provided with some mechanism for the rapid escape of the gas. When blood is exposed to reduced pressure, carbon dioxide is released in two ways: (i) rapidly from the carbonic acid, and (ii) slowly from the acid carbonates in solution. The speed of the first reaction led Meldrum and Roughton to suspect the existence of a catalyst, which they later isolated from the red corpuscles, and named **carbonic anhydrase** because it catalysed the reversible reaction,



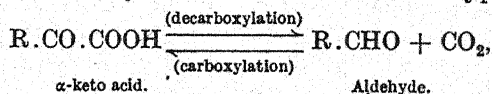
When blood is previously treated with dilute cyanide or other appropriate enzyme inhibitors, and then exposed to a vacuum, the amount of rapidly-released carbon dioxide is greatly but not entirely diminished, whereas the slowly-released carbon dioxide is unaffected.

From this it is concluded that carbon dioxide is transported in the blood in three distinct forms: (a) as stable acid carbonate, (b) as unstable carbonic acid, readily dehydrated by an enzyme, and (c) as an unstable salt, not attacked by the enzyme. This salt has been identified as a carbamate produced by union between CO_2 and an uncharged $-\text{NH}_2$ group, such as occurs in haemoglobin in slightly alkaline solution.



Carbamino
derivative

Carbon Dioxide Assimilation.—Green plants and a few specialised pigmented bacteria readily assimilate free CO_2 as their chief source of carbon, and subsequently elaborate it into organic compounds by means of light energy. CO_2 is also assimilated, though to a much lesser extent, by many other cells and tissues, including those of the higher animal. Thus, by a reversible reaction of the type:



α -keto acid.

Aldehyde.

CO_2 can re-enter the metabolic cycle.

A converse process is responsible for the liberation of CO_2 in plant and animal respiration.

IONIC SYSTEMS

Ions are electrically charged particles formed by the spontaneous dissociation of acids, bases and salts, when dissolved in water or

other ionising solvents. Ions may be classified according to sign as : (i) positively-charged *cations*, R^+ , which migrate to the cathode, or negative electrode ; (ii) negatively-charged *anions*, R^- , which migrate to the anode, or positive electrode ; and (iii) doubly charged dipolar, or *zwitter*-ions, $+R^-$, which, when their charges are equal, migrate to neither electrode.

The principal biological ions are :—

- (1) *Metallic cations* : Na^+ , K^+ , Ca^{++} , Mg^{++} .
- (2) *Non-metallic cations* : H^+ , NH_4^+ , dipolar ions in acid solution.
- (3) *Non-metallic anions* : Cl^- , HCO_3^- , $H_2PO_4^-$, aliphatic and other acid radicles ($R.COO^-$), dipolar ions in alkaline solution.
- (4) *Dipolar ions* : Proteins and amino acids ($+H_3N.R.COO^-$).

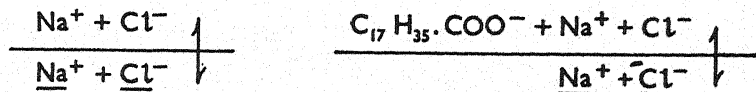
The other biological elements, such as Mn, Fe, Cu and I, occur either as compounds or in very low ionic concentration.

The ionic composition of the tissues and tissue fluids determines : (a) conductivity, (b) buffering power and pH, (c) oxidation-reduction conditions, (d) membrane potential and, in part, (e) osmotic pressure.

Chloride Shift.—Hamburger observed that when carbon dioxide enters the blood there is an accompanying migration of Cl^- from plasma to corpuscles. Conversely, when carbon dioxide escapes from blood there is a migration of Cl^- from corpuscles to plasma, although this change takes place against the concentration gradient for Cl^- . In outline, this "chloride shift" is due to (a) diffusion of carbonic acid into the corpuscle, where it is ionised by the intracellular buffer systems, which accept H^+ from the acid ; (b) accumulation of HCO_3^- ions within the corpuscle until their concentration exceeds that of the HCO_3^- ions in the plasma ; (c) diffusion of HCO_3^- ions from corpuscle to plasma, where they displace a corresponding number of Cl^- ions, which enter the corpuscle to balance the H^+ ions derived from the carbonic acid.

The Donnan Membrane Effect.—When a simple electrolyte, such as sodium chloride, is separated from its solvent by a permeable membrane, ionic diffusion takes place until equilibrium is reached, when the product of the concentrations of the ions on either side of the membrane is the same, or $[Na^+] \times [Cl^-] = [Na^+] \times [Cl^-]$. Furthermore, by analysing samples from each side, it can be shown that the sodium concentration $[Na^+]$ on one side equals that on the other side of the membrane, $[Na^+]$, and also that $[Cl^-]$ equals $[Cl^-]$. If, however, a non-diffusible ion, such as a stearic acid radicle, $C_{17}H_{35}.COO^-$, be present on one side of the membrane it will inhibit the migration of some of the ions of the opposite sign,

in this instance, Na^+ . When equilibrium has set in, $[\text{Na}^+] \times [\text{Cl}^-] = [\text{Na}^+] \times [\text{Cl}^-]$, as before; but now $[\text{Na}^+]$ is greater than $[\text{Na}^+]$ and consequently $[\text{Cl}^-]$ must be greater than $[\text{Cl}^-]$.



Simple equilibrium

$$[\text{Na}^+] = [\underline{\text{Na}^+}]$$

Complex equilibrium

$$[\text{Na}^+] > [\underline{\text{Na}^+}] \quad [\underline{\text{Cl}^-}] > [\text{Cl}^-]$$

This unequal concentration of the ions leads to a difference in potential between the solutions on either side of the membrane, which can be calculated or estimated electrometrically. The phenomenon was first predicted and experimentally verified by F. G. Donnan, in 1911, and is generally known as the Donnan Effect. It has been applied to the study of protein systems by J. Loeb.

The physiological consequences of membrane potentials and ionic displacements are very great, and these processes modify or determine many forms of activity, including muscle contraction, nerve conduction, gland secretion, as well as general cell growth.

Once a cell has reached maturity it is, as a rule, impermeable to certain ions, and depends on its ionic inheritance for the discharge of many of its functions. These, in turn, are regulated by the ionic pattern of the environment, the constituents of which enter into various synergic and antagonistic combinations whereby their physiological properties are enhanced or depressed. Thus, a balance operates between the four ions, Na^+ , K^+ , Ca^{++} and Mg^{++} , which may be written:—

$$\begin{array}{c} \text{Na}^+ \\ \text{K}^+ \end{array} \longleftrightarrow \begin{array}{c} \text{Ca}^{++} \\ \text{Mg}^{++} \end{array}, \text{ or, } k = \frac{[\text{Na}^+] + [\text{K}^+] + [\text{OH}^-]}{[\text{Ca}^{++}] + [\text{Mg}^{++}] + [\text{H}^+]}$$

Alteration in the concentration of any one ion, within limits, can be balanced by alteration in the concentration of one of the other ions, and as long as the coefficient k is unchanged the physiological properties of the solution will remain unaltered, at least as regards its effect on a particular tissue. Irritability increases with k .

The recognition of metallo-ionic balance began with the work of Ringer on the maintenance of the heart beat by perfusion.

For the mammalian heart, a perfusion fluid must contain approximately: NaCl , 0.9 per cent.; KCl , 0.04 per cent.; CaCl_2 , 0.024 per cent.; and NaHCO_3 , 0.01–0.03 per cent. This mixture constitutes Locke's solution.

Na^+ maintains the beat.

Ca^{++} affects the contraction process, and if excessive causes a decrease in relaxation, and ultimate cessation in a state of extreme contraction or "calcium rigor."

K^+ is antagonistic to Ca , and if excessive causes a weakening of the beat, and cessation in a state of complete relaxation. Cl^- and Na^+ maintain the osmotic pressure of the fluid. NaHCO_3 forms a buffer system to keep the pH on the side of slight alkalinity. Rise in $[\text{H}^+]$ causes increased relaxation and weakening of beat; fall in $[\text{H}^+]$ causes decrease in relaxation.

At present it is only possible to apply the principle of ionic balance in a general way when several different species of ions are concerned, but it has been worked out more fully for growth conditions in many land and water plants (Osterhout, 1906-1936).

Anion-Cation Balance.—All tissues, secretions and mixed salt solutions contain a variety of electro-negative anions and electro-positive cations, and, however the composition may alter, the total number of ionic positive charges is balanced by an equivalent number of negative charges. If this were not so, the solution would be positively or negatively charged with respect to its environment. Electrolyte unbalance may occur locally during secretion, muscle-contraction, and the passage of a nerve impulse, and the resulting changes in electric potential can be recorded. A specialised example is seen in the electric organ of some fish, which is a modified form of muscle tissue capable, in the *Torpedo*, or *Stinging Ray*, of developing a brief potential of about 200 volts, when stimulated.

Electrolyte balance was formerly termed *acid-base* balance, in accordance with the old classification of all cations as acidic and all anions as basic. It is measured usually in terms of *milligram-equivalents*, *M.Eq.*, or *milli-equivalents* of the ions present, the values being obtained by dividing the equivalent weight, in mg., of each species of ion, by the valency of the ion.

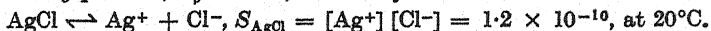
Thus, a Ca^{++} concentration of 1 mol denotes 40 gm. Ca^{++} per litre, while a concentration of 1 *equivalent* is 40/2 gm. per litre. Similarly, a *milli-mol* concentration of Ca^{++} is 40 mg. per litre, and a *milli-equivalent* concentration is 20 mg. per litre, since Ca has a valency of 2.

Anion-Cation Balance in Average Sea Water

	Na^+	K^+	Ca^{++}	Mg^{++}	Cl^-	CO_3^{--}	SO_4^{--}	PO_4^{--}
Gm./Kgm.	11.0	0.4	0.4	1.3	19.0	0.09	2.7	0.015
M.Eq./Kg.	480	10	20	108	535	18	56	0.16
Total cation charge	618				Total anion charge			
					609			

The calculation of anion-cation balance provides a check on chemical analysis. If the total charges do not balance, it shows that some species of ion has been overlooked, or wrongly estimated.

Solubility Product.—When the solubility is less than 0.01 gm. molecule per litre, a salt is described as "sparingly soluble." In saturated solutions, the product of the total concentrations of the ions is a constant value for a given temperature, and is termed the *solubility-product*, S_p . Thus, for the system :



Where S_{AgCl} is the solubility-product of silver chloride, and $[\text{Ag}^+]$ and $[\text{Cl}^-]$ are measured in gm. ions per litre, and represent a solubility of about 1.5 mg. AgCl per litre.

A solution is not fully saturated until the product of the concentration of the ions reaches the value of the solubility-product of the parent salt. When this is exceeded, the solution is supersaturated and the salt tends to precipitate. Precipitation thus can be effected by increasing the concentration of either ion until the value of the S_p is reached. Solubility-products are of interest in animal biochemistry in regard to the deposition of calcium phosphate and carbonate during bone-formation, and the production of renal calculi.

Representative values for S_p at 20°C . are: CaF_2 , 3.2×10^{-11} ; BaSO_4 , 1.2×10^{-10} ; CaC_2O_4 , 3.8×10^{-9} ; CaCO_3 , 1.7×10^{-8} ; MgCO_3 , 2.6×10^{-5} ; FeS , 1.5×10^{-19} ; $\text{MgNH}_4\text{.PO}_4$, 2.5×10^{-13} .

The Inland Sea.—In 1889, Bunge, the Swiss chemist, outlined a theory to explain the occurrence and distribution of the biological metals. Life, he assumed, arose in the tepid waters of a primæval ocean, and during the slow evolution of the invertebrates and primal vertebrates the tissues became adapted to a saline environment. When life eventually migrated from sea to land, the later vertebrates carried the chemical legacy of a marine ancestry. In the ocean the concentration of sodium chloride exceeds that of any other solute; consequently, it is the chief inorganic constituent of the tissue fluids of the animal. The theory was developed by Quinton (1897, 1912), who assumed, somewhat hastily, that all the inorganic constituents of blood-plasma and sea water were the same in nature and relative proportions, and that sea water when diluted until isotonic with the plasma of the animal is the correct physiological saline. That is to say, the internal medium of life is not water, but sea water—a concept anticipated with more magnificence by Algernon Swinburne :—

“—With heart's thanksgiving
That in my veins like wine
Some sharp salt blood of thine,
Some springtide pulse of brine
Yet leaps up living.”

In 1904, Macallum independently reached the conclusion that "the blood-plasma of vertebrates and invertebrates with a closed circulatory system is, in its inorganic salts, but a reproduction of the sea-water of the remote geological period in which the prototype representative of such animal forms first made their appearance."

He supported his conclusion by a series of analyses of blood-serum and sea-water :—

Solution.	Percentage of Element.				
	Na	Ca	K	Mg	Cl
Sea water . . .	1.072	0.042	0.038	0.136	1.932
Human serum . .	0.302	0.009	0.020	0.002	0.389
Sea water, diluted 1 : 3	0.357	0.014	0.012	0.045	0.644

The marked difference between the high concentration of magnesium in sea water and its low concentration in all vertebrate tissue fluids is explained by Macallum as being due to the selective action of the kidney, which stabilised the composition of the tissue fluids at a time when the magnesium content of the ocean was less than its present value.

Macallum's conception of a palæochemical factor at work in the organism to-day has been criticised by Dakin (1931) and by Pantin (1931).

Dakin observes that the composition of a tissue fluid represents the combined effects of a number of physiological processes, that in turn depend on the existence of membranes separating the cells from the internal and the external environments: "We can only accept the universal saline composition of the blood of *metazoa*—with its variations—as a possible reflection of a long-continued existence and probable origin in a saline medium."

If all the body chloride exists in true solution as Cl^- , it can be calculated that extracellular water makes up 26–28 per cent. of human body weight; intracellular water varies from 29 to 45 per cent., being lower in obese subjects.

Osmotic difference between the body fluids and the environment is maintained in order to preserve the inorganic pattern of the cells, and probably depends more on the requirements of cytoplasmic metabolism than on the conditions imposed by an immemorial marine ancestry.

The composition of the ocean in relation to organic evolution has been discussed in detail by Conway (1943).

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CHAPTER 4

SOLUTIONS AND COLLOIDAL SYSTEMS

ALL the chemical reactions associated with life take place between substances in aqueous solutions and aqueous colloidal systems, and the properties of these solutions and systems largely determine tissue organisation and function.

Solutions are micro-homogeneous mixtures the composition of which may vary within certain limits of saturation.

The characteristic of a micro-homogeneous mixture is that its constituents cannot be separated by mechanical methods, such as sedimentation or filtration. In the nomenclature of physical chemistry, a true solution is *monophasic*, all its constituents being present as part of the same physical state, or *phase*.

The constituent present in excess is termed the *solvent*, the other constituents being the *solutes*. In a true solution the solvent is usually a liquid, but may be a solid, as in the so-called "solid solutions" represented by glasses and alloys. The solutes, or dissolved constituents, may be gases, liquids or solids, the ultimate state of the solution (liquid or solid) being determined by the state of the solvent. The composition of a solution is limited by the solubility of the solutes, a critical point being reached when the solution is saturated in regard to a particular solute, the excess of which remains undissolved or separates out from the saturated solution, and may be removed by sedimentation or filtration. These heterogeneous mixtures are said to be *polyphasic* in that they contain mechanically-separable forms of matter.

Structure.—The solute particles in a solution may be present as non-ionised molecules, such as sugar, or as electrically charged ions, three types of which exist: (i) positively charged cations, such as H^+ , Na^+ , and Ca^{++} ; (ii) negatively charged anions, such as OH^- and Cl^- ; and (iii) dipolar, or zwitter-ions, such as amino acids, $+R^-$, which carry charges of opposite electric sign.

Conductivity.—The electrical conductivity of the solution is determined by the nature and concentration of the solute particles. Solutions of non-ionised molecules or dipolar ions have minimal conductivity and are termed non-electrolytes; solutions containing ions have a conductivity depending on the ionic concentration, and are termed electrolytes.

All aqueous solutions have a low residual conductivity irrespective of the nature of the added solutes, owing to the fact that water itself

is slightly ionised into H^+ and OH^- , and thus generates ionic solutes.

Properties of Solutions.—In addition to the properties due to the solvent and the solutes, solutions display characteristics which may be described as emergent, since they are not shown by the individual constituents; of these, the most familiar is osmotic pressure.

Osmotic Pressure.—Solution is a spontaneous process and tends to occur whenever a solute is added to an appropriate solvent. If, however, a solute or a concentrated solution be separated from a solvent by means of a *semipermeable membrane* the tendency of the solute particles to dissolve is shown by the pressure they exert on the membrane. This force, or *osmotic pressure*, can be measured in various ways, and affords information as to the number of solute particles present in a given volume of solution.

Semipermeability implies that a surface allows the passage of the solvent but restricts the passage of the solute responsible for the osmotic pressure. Semipermeability varies greatly with material structure. A filter paper is permeable to all solutes present in true aqueous solution, and also permits the passage of colloidal mixtures, such as milk and egg albumin. An animal membrane, such as parchment or peritonæum, will restrict the passage of proteins, soaps and other solutes of large molecular dimensions. Thin membranes can be constructed of collodion, cellophane, or gelatine of such degree of selectivity as to be permeable to ions but not to simple molecules.

Estimation of Osmotic Pressure.—(i) *Direct.*—The solution of known concentration is enclosed in a semipermeable container attached to a manometer tube. The container is immersed in a vessel containing an excess of the solvent, some of which diffuses into the container causing a rise of the solution in the manometer tube. When equilibrium is reached, the osmotic pressure of the diluted solution inside the container is balanced by the hydrostatic pressure of the liquid in the manometer, and can be measured in atmospheric units. Modifications of the apparatus include the use of a mercury manometer to avoid excessive dilution of the mixture, and the employment of metallic containers with frames to support the membranes.

(ii) *Indirect.*—The boiling point of a solvent is raised and the freezing point is depressed by addition of solutes, the extent of the change being proportional to the concentration of solute particles.

It can be shown from the gas laws that an aqueous solution of a non-ionising solute in molecular concentration (1 gm. molecular equivalent of solute in 1 l. of solution) has an osmotic pressure of 22.4 atmospheres at 0° , a boiling point of 100.52° , and a freezing point of -1.86° . Consequently, if the molecular weight of a non-

ionising solute, such as a sugar, is known, it is possible to calculate the osmotic pressure, boiling point and freezing point of a solution of any given concentration.

Similarly, knowing the concentration of the solute it is possible to calculate the molecular weight from any one of the three properties of the solution. In practice, the easiest estimation is that of the freezing point, which is determined in a *cryoscope*, in which the value of Δ , the depression of the freezing point, is read by means of a sensitive Beckmann thermometer.

$$\text{Osmotic pressure, in atmospheres} = \frac{22.4 \times \Delta, \text{ in } ^\circ\text{C}}{1.86}.$$

The molecular weight, M , of a single solute can be calculated from the depression of the freezing point, Δ , of a solution containing w gm. of solute per litre, since

$$M = \frac{1.86 \times w}{\Delta}.$$

Solute.	M .	Osmotic Pressure of a 10 per cent. Solution.	Freezing Point of a 10 per cent. Solution.
Urea . . .	60	37.34 atm.	-3.1°
Glucose . . .	180	12.4 "	-1.03°
Fructose . . .	180	12.4 "	-1.03°
Sucrose . . .	342	6.5 "	-0.54°

The osmotic pressure of the blood plasma and tissue fluids is determined by a variety of solutes, the chief of which are Na^+ and Cl^- , and is maintained in the region of 7 atmospheres (corresponding to a freezing point of -0.56°) by the intake of water and the excretion of urine, the osmotic pressure of which may vary between 12 and 24 atmospheres during the day.

Milk, being a true secretion, has a constant freezing point range of -0.53° to -0.57°C .

COLLOIDAL SYSTEMS

Intervening between true solutions and unstable suspensions, which can be separated by simple mechanical methods, is a class of solutions termed *colloidal* by Graham, in 1856, to denote their glue-like non-crystallisable nature, as distinct from that of the *crystalloids*, or solutes present in true solutions. By selective *dialysis*, or filtration through parchment, he was able to separate colloids from accompanying diffusible crystalloids. Subsequently, it was found that the intrinsic difference between the two groups

depended on the size of the solute particles and not on the crystallisability, and Ostwald suggested that colloids represented a state of matter rather than a class of compounds. In a true solution, the solute particles are less than $1\text{ m}\mu$ in diameter; in a colloidal solution the particles range from $1\text{ m}\mu$ to $100\text{ m}\mu$ in diameter; in suspensions and emulsions the particles are greater than $100\text{ m}\mu$ in diameter.¹

Colloidal solutions resemble true solutions in that they are homogeneous and are not resolved by simple filtration or sedimentation. The dispersed particles, however, display surface properties not shown by true solutes, and also may be separated by special methods involving the high-speed centrifuge or by ultra-filtration, and for this reason colloids are sometimes termed polyphasic solutions, the term phase being used to denote a mechanically separable form of matter.

To emphasise the distinction, the particles in a colloidal system are termed the *disperse* or *internal phase*, the solvent being the *continuous* or *external phase*.

Classification of Colloidal Systems.—Unlike true solutes, the various components of a colloidal system can be any one or more of the three states of matter.

Representing the system as *solute/solvent*, the following arrangements are possible: gas/liquid, gas/solid, liquid/gas, liquid/liquid, liquid/solid, solid/gas, solid/liquid, solid/solid.

(i) *Gas/liquid* systems are represented by foams and froths, the permanence of which depends on the presence of stabilisers, such as soaps and saponin glucosides, which form froths when their aqueous solutions are shaken up with air.

Froth production is rare in nature, apart from fermentations, but an interesting example is afforded by the plant parasite that protects itself by means of a foam derived from leaf sap. Pathological and often fatal examples of froth formation in the higher animal are seen in air embolus, and pulmonary cedema.

(ii) *Gas/solid* systems in which gaseous particles are distributed through a solid external phase are of industrial importance in connection with the adsorption of vapours by means of charcoal filters, and their study has been encouraged by the introduction of gas as a weapon in civilised warfare. Many porous substances, including spongy platinum and palladium, adsorb and subsequently dissolve gases.

(iii) *Liquid/gas* systems, represented by mists and fogs, occur temporarily when air saturated with moisture is cooled suddenly. The stability of a mist depends on the size of the particles, being

¹ $1\text{ mm.} = 1,000\text{ }\mu$ (microns). $1\text{ }\mu = 1,000\text{ m}\mu$ (millimicrons).

greatest when they are small, but stabilising factors, such as products of coal combustion, also operate to preserve the characteristic atmosphere of industrial towns in damp weather.

Cigarette smoke provides an example of two colloidal systems. The bluish smoke from the combustion is a dispersion of carbon particles carried upwards by the current of heated gas. The brownish exhalation is a fog formed by salivary moisture stabilised by products of tobacco combustion.

(iv) *Liquid/liquid* systems are termed *emulsoids* to distinguish them from unstable emulsions. They are an important class of biological colloids, and represent the form in which liquid fats are transported or secreted by the organism. Milk is a 3-4 per cent. solution of fat, which is in an emulsoid form stabilised by milk protein.

(v) *Liquid/solid* systems, or *gels*, are represented by natural and artificial jellies and mucoids, and by butter, all of which consist of a solid external phase retaining a dispersed liquid phase in its interstices.

(vi) *Solid/gas* systems are represented by colloidal smoke, the stability of which depends on the fineness of the particles and the presence of stabilisers.

(vii) *Solid/liquid* systems, sols or *suspensoids*, constitute the principal biological colloids, and are represented by the soluble proteins of the tissues and tissue fluids, and similar compounds of high molecular weight.

Metallic sols of gold, silver, copper, etc., form an important class of suspensoids, and are used industrially and therapeutically in many ways.

(viii) *Solid/solid* systems are often included among true solid solutions, but are of little biological interest, although it is possible to attribute the structure of bone and other metallo-skeletal tissue to the formation of solid solutions from calcium salts.

Properties of Colloidal Systems.—Colloidal solutions resemble true solutions in that they exert an osmotic pressure and have a lower freezing point than that of the solvent. Neither effect is as marked as in the true solution owing to the relatively greater size and smaller concentration of the dispersed particles. The osmotic pressure of the plasma proteins, however, is an important factor in maintaining the volume of blood, and in man has a normal value of 305-307 mm. H_2O (0.03 atmospheres) at 22° C. This, though low, is effective, because the plasma proteins are unable to escape from the vascular system, and thus tend to retain tissue fluid.

(1) *Faraday-Tyndall Effect.*—When a convergent beam of light is sent transversely across a true solution, and examined by an

observer at right-angles to the path of the beam, the solution appears optically empty. When the observation is repeated using a colloidal solution, light is reflected from the surface of the disperse particles, and the path of the beam is seen as an illuminated cone. By using a strong source of light, colloidal particles can thus be detected in very low concentration.

A similar method of transverse illumination is used in the ultra-microscope whereby particles smaller than the shortest wave-length of visible light (400 $m\mu$) are revealed as radiating points against a dark background.

When strongly illuminated by light of short wave-length, it has been found that molecules in true solution are able to scatter sufficient of the radiation to enable their structure to be studied spectroscopically. This *Raman effect* is employed in the elucidation of molecular architecture.

(2) *Brownian Movement*.—When examined microscopically against a dark background, colloidal particles are observed to be in continual rapid irregular motion, a phenomenon first recorded in 1827 by the botanist R. Brown. Brownian movement is a form of perpetual motion, and is due to bombardment of the disperse particles by the molecules of the solvent.

(3) *Migration in an Electric Field, Electrophoresis*.—Dispersed particles carry an electric charge, and migrate to the anode or the cathode when the solution is electrolysed. Typical positively charged colloids (cations) are: proteins in acid solution, hæmoglobin, ferric hydroxide and aluminium hydroxide suspensoids. Typical negatively charged colloids (anions) are: proteins in alkaline solution, starch, soaps and metallic sols.

The colloidal charge is altered by addition of electrolytes, especially acids or bases, and may be neutralised or reversed owing to combination between the colloid and solute ions. A neutral disperse phase is said to be iso-electric, in which condition it migrates neither to anode nor to cathode.

(4) *Adsorption at the Phase Interface*.—Solutes that lower surface tension tend to accumulate on the free surfaces of colloidal particles. This surface condensation is termed adsorption, and is of special significance in colloidal systems because of the enormous area presented by the disperse phase. Colloids often display preferential absorption for a particular class of solutes, and one solute may compete with another solute and displace it from the adsorbing surface.

This is exemplified in the use of tartrazol as an adsorption indicator in the estimation of chlorides (p. 457).

As a result of adsorption, the solute, or *adsorbate*, becomes highly concentrated on the colloid surface, and may undergo subsequent

changes due to chemical reaction with the colloid. Adsorption is a preliminary stage in enzyme catalysis, and is of primary importance in regulating the distribution of solutes among the tissue surfaces that make up the framework of structures associated with life.

Colloidal Stability.—The stability of a colloidal system depends on the combined effect of four factors :—

(1) *Diameter of Disperse Particles.*—The sedimentation rate of a sphere falling in a liquid may be found by means of Stokes's law :

$$V = \frac{2 r^2 (s - s') g}{9 u}$$

where V = rate of fall,

r = radius of particle,

s = density of particle,

s' = density of continuous phase,

g = gravity constant (981),

u = viscosity of solution.

From this it will be seen that rate of sedimentation is proportional to the square of the radius, and if the particle be sufficiently small, months, years, or even centuries, will be required for complete sedimentation.

(2) *Brownian movement* tends to keep the particles distributed throughout the system.

(3) *Electric surface charge* tends to keep the particles from flocculating and precipitating, since bodies of like charge are mutually repellant. The importance of the surface charge is shown by the fact that colloids are least stable when at the isoelectric point.

(4) *Surface stabilisation.* Metallic sols, although they have a disperse phase of very small dimensions, are so unstable that they are classified as *lyophobic*, owing to the readiness with which they lose their solvent phase. Emulsoids and organic suspensoids, on the contrary, are examples of *lyophil* or hydrophil colloids, and are usually very stable. The difference is attributed to adsorption of the solute by the lyophil particles (M. Fischer, 1933).

Colloid Stabilisers.—These are substances which when added in small quantities promote the formation of colloidal systems and render them less liable to spontaneous precipitation. Gum acacia, gum tragacanth, soaps, saponins, gelatin and lecithin are representative stabilisers used industrially. Egg yolk, on account of the lecithin it contains, is used in pharmaceutical and in domestic preparations, such as the manufacture of mayonnaise by emulsification of vinegar in oil.

Precipitation and Coagulation of Colloids.—Any operation that removes the stability factors in a colloidal system tends to bring

about aggregation of the disperse particles. Violent agitation, as in the churning of milk, addition of colloids or electrolytes of the opposite electric charge, freezing and thawing, all promote precipitation of colloidal particles. An important example is the irreversible coagulation that takes place when certain higher proteins are heated in slightly acid solution (p. 145).

Analysis of Colloidal Systems.—The average dimension of the disperse phase particles may be found in several ways, the most important of which are :—

(1) *Ultra-filtration* through surfaces of standard porosity. The filters usually employed are collodion, cellophane and gelatin. From the strength of a gelatin filter the size of the pores may be calculated, and depend on the concentration of gelatin present, irrespective of the thickness of the filter.

(2) *Rate of sedimentation* may be measured by means of a sedimentation balance, one pan of which is immersed in the solution, and acquires weight from the subsidence of the particles. The radius of the particles is then calculated by means of Stokes's law. The method is only applicable to suspensions and coarser suspensoids that subside rapidly.

Average Diameter of Dispersed Particles

Type.	Visibility.	Example.	Diameter.
Molecular	Not visible by ultra-microscope	True solutes	0.5 m μ —5 m μ
Ultra-microscopic	Not visible by microscope	Colloidal solutes	5 m μ —100 m μ
Microscopic	Not visible by the unaided eye	Fine suspensions	200 m μ —1 μ
Directly visible		Coarse suspensions	> 10 μ (0.01 mm.)

0.1 m μ .	1 m μ .	10 m μ . 100 m μ .	1 μ .	10 μ .	100 μ . 1 mm. (1,000 μ).
True solutions		Colloids	Suspensions		Precipitates
Particles pass through ordinary filter paper			Particles retained by ordinary filter paper		
Brownian movement			No Brownian movement		

(3) *Ultra-centrifugal Methods.* By means of a high speed centrifuge rotating at 10,000 to 400,000, or more, revolutions per minute, it is possible to submit colloidal systems to forces sufficient to separate the dispersed phase. The sedimentation rate is found by observation of the meniscus of separation, and from this the radius of the particles is calculated. The method has been used successfully by Svedberg for the estimation of the diameter and weight of protein molecules (p. 158).

Methods of Preparing Colloidal Solutions.—(1) *Use of a suitable solvent.*—Many organic compounds, such as proteins, soaps, and gums, form colloidal solutions spontaneously when treated with water, and occur as colloidal systems in their natural conditions.

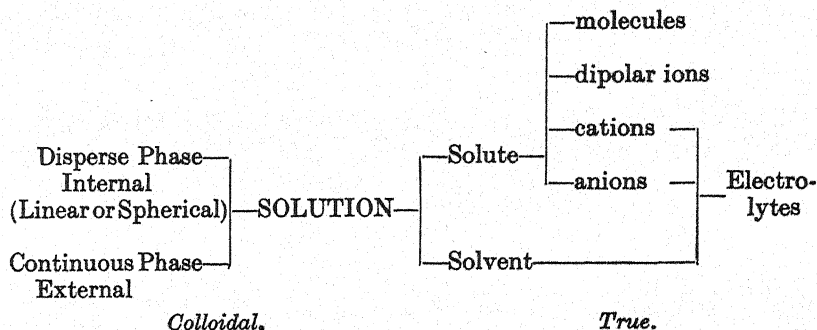
(2) *Condensation of True Solute Particles.*—By reduction or hydration it is possible to aggregate the ions of various metals so that they form particles of colloidal dimension and pass from the true to the colloidal state.

(3) *Dispersion, or Peptisation, of Insoluble Particles.*—By mechanical disintegration in a colloid mill it is possible to render insoluble substances, such as graphite or chalk, sufficiently fine to form colloidal solutions in water, oil and other liquids, while it is also possible by means of an electric arc formed between electrodes of a metal, immersed in water or other liquids, to disperse the metal in the form of a sol. These are industrial methods used for preparing colloidal lubricants and therapeutic agents.

Colloidal particles can be classified according to shape as linear and spherical. Linear colloids, such as cellulose, are fibrous, and dissolve with much swelling to form viscid solutions. Spherical colloids, such as glycogen, are powders in the solid state, and give solutions of low viscosity.

Summary

True Solutions.	Colloidal Solutions.
1. One phase (mechanically homogeneous).	More than one phase (mechanically heterogeneous).
2. Optically empty. No Faraday-Tyndall cone.	Optically dense. Faraday-Tyndall cone when illuminated transversely.
3. Solute particles less than 1-5 $m\mu$ in diameter.	Disperse particles range from 5 $m\mu$ to 100 $m\mu$.
4. No Brownian movement.	Particles show Brownian movement.
5. Solute particles show no surface properties other than the Raman effect.	Disperse particles show surface pro- perties and carry a surface charge.



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PART II

ORGANIC BIOCHEMISTRY

"Bear with me, gentlemen, while I remind you
of the incessant molecules that bind you."

HUMBERT WOLFE.

CHAPTER 5

CLASSIFICATION AND CHARACTERISTICS OF ORGANIC COMPOUNDS

WHILE the greater part of the tissues and tissue fluids of the organism is inorganic in that it is composed of water and dissolved salts, the colloidal matrix of the cells, secretions and vascular fluids is organic in that it is made up of compounds containing carbon and hydrogen as structural elements. In addition, the manifold chemical transformations and exchanges that reveal the operations of life are concerned chiefly with the decomposition, elaboration, storage and excretion of organic compounds.

Living organisms may be divided into two classes : the *autotrophes*, represented by green plants and some highly specialised bacteria ; and the *heterotrophes*, represented by animals and by plants free from chlorophyll. Autotrophic organisms are able in the presence of sunlight to synthesise their organic constituents from an inorganic environment providing water, carbon dioxide, nitrate, ammonia and the additional biological elements. Heterotrophic organisms require organic food materials as well as water, oxygen and the biological elements. In the collaboration that has made animal life possible on earth, the autotrophes act as collectors and storers of energy in chemical form available for the nutrition of the heterotrophes.

The bio-organic compounds associated with plants and animals are wide in variety and often great in complexity, and no classification yet proposed is entirely logical. Features of chemical structure, physiological significance and biological distribution are selected according to convenience.

Classification by Structure.		Classification by Function.	
1. Carbohydrates.	8. Carotinoids.	1. Plastics.	7. Buffers.
2. Proteins.	9. Terpenes.	2. Nutrients.	8. Storage products,
3. Lipides.	10. Flavins.	3. Hormones.	etc.
4. Steroids.	11. Flavones.	4. Catalysts.	
5. Porphyrins.	12. Alkaloids,	5. Vitamins.	
6. Purines.	etc.	6. Pigments.	
7. Pyrimidines.			

Neither of these classifications is exclusive nor exhaustive, and each merely serves to emphasise the biological significance of the included compounds.

ORGANIC TYPE FORMULÆ

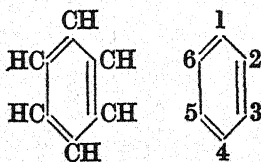
Organic compounds, other than symmetrical hydrocarbons, can be represented as being composed of a linear or cyclic carbon nucleus or *radicle*, R, carrying one or more reactive groups or side-chains. The radicle confers stability and physical characteristics on the compound; the side-chains determine chemical reactivity. Cyclic radicles are represented chiefly by phenyl, C_6H_5- , and indolyl, C_8H_7N- , rings, neither of which can be assembled by the higher animal, but must be provided in the diet. Reactive groups are represented chiefly by hydroxyl, $-OH$, carboxyl, $-COOH$, aldehyde, $-CHO$, keto, $=CO$, and amino, $-NH_2$, configurations.

(1) Systems Present in Biological Compounds.

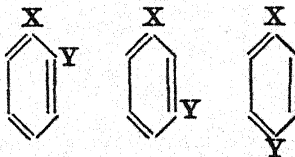
(a) Derivatives of Open-Chain, or Linear Hydrocarbons.

Parent Hydrocarbon.	Radicle.
Methane, CH_4	Methyl, <i>Me</i> , CH_3- , Methylene, $CH_2=$
Ethane, C_2H_6	Ethyl, <i>Et</i> , $CH_3.CH_2-$, Vinyl, $CH_2:CH-$
Propane, C_3H_8	Propyl, <i>Pr</i> , $CH_3.(CH_2)_2-$
	Allyl, $CH_2:CH.CH_2-$
Butane, C_4H_{10}	Butyl, $CH_3.(CH_2)_3-$
Pentane, C_5H_{12}	Amyl, $CH_3.(CH_2)_4-$
Hexane, C_6H_{14}	Hexyl, $CH_3(CH_2)_5-$

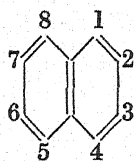
(b) Derivatives of Closed-Chain, or Cyclic Hydrocarbons.



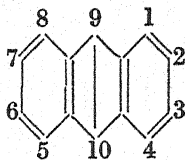
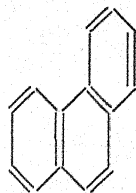
Parent Hydrocarbon
Benzene, C_6H_6



ortho (1:2), meta (1:3) para (1:4)
Disubstitution Derivatives.

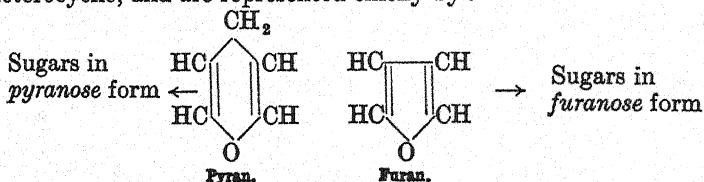


Naphthalene.

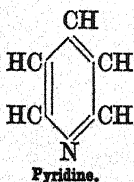
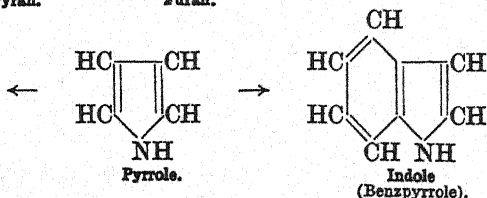
Anthracene.
Parent Polycyclic Hydrocarbons.

Phenanthrene.

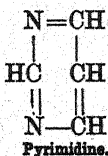
(c) *Heterocyclic Compounds*.—Cyclic structures containing other elements in the ring in addition to carbon and hydrogen are termed heterocyclic, and are represented chiefly by :—



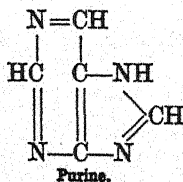
Porphyryns
Blood pigments
Bile pigments
Chlorophyll



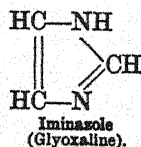
Pyridine.



Pyrimidine.



Purine.

Iminazole
(Glyoxaline).

Groups and Linkages Present in Biological Compounds.

Primary alcohol, $-\text{CH}_2.\text{OH}$

Secondary alcohol, >CH.OH

Amino, $-\text{NH}_2$ attached to
 $-\text{CH}_2-$ or $=\text{CH}-$

Amido, $-\text{NH}_2$ attached to
 $-\text{CO}-$

Carbamido, or uramido,
 $-\text{NH.CO.NH}_2$

Guanidino, $-\text{NH.C(NH).NH}_2$

Aldehyde, $-\text{CHO}$

Carboxyl, $-\text{COOH}$

Keto, $:\text{CO}$; Imino, $:\text{NH}$

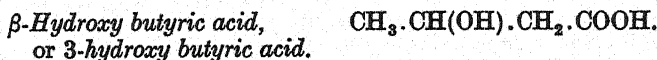
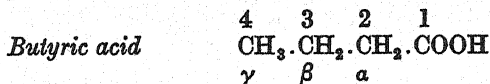
Ether linkage,

Ester Linkage,

Disulphide linkage,
 $-\text{CH-S-S-CH}_2-$

Thiol, or sulphhydryl group, $-\text{SH}$

The position of a substituent group in a linear compound is denoted by numbering the C atoms from right to left, or by the use of a Greek alphabet, the C atom next the characteristic group in the compound being described as the α -carbon. Confusion is liable to arise between the two types of nomenclature, and the numerical form is preferable.



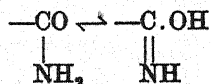
The position of a group in a cyclic compound is denoted numerically in reference to a particular carbon atom, C¹.

Double bonds, —C=C— , in organic compounds are denoted by the symbol Δ with a pair of suffixes to indicate the position number of each C atom linked by the double bond.

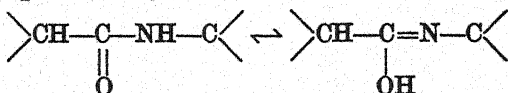
The chief reactions undergone by organic compounds include *oxidations*, *reductions*, *condensations*, or union by elimination of water, *hydrolyses*, or resolution by addition of water, *polymerisation*, or combination of two or more similar molecules, *internal rearrangement* and *ring formation*.

Tautomerism, or dual configuration, is shown by certain organic groups and compounds, the structure of which differ under different conditions. Important biochemical examples are :—

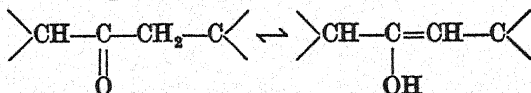
(a) *The Amide Group,*



(b) *The Peptide Linkage,*

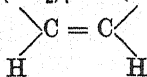


(c) *The Keto Compounds,*

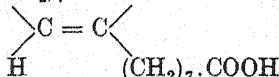
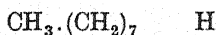


Mesomerism, or chemical resonance, is said to occur when a substance exists in an intermediate or *hybrid* state not capable of being exactly represented by a single structural formula.

Cis-trans Isomerism.—Rotation can occur at a double-bond linkage, giving rise to a geometrical isomer.



Oleic Acid
(*Cis*-oleic acid)
m.p. 13–14° C.



Elaidic Acid
(*Trans*-oleic acid)
m.p. 45° C.

In the *cis*-isomer, the atoms or groups on one side of the horizontal axis are the same. Where more than one pair of double linkages occur in the molecule, more complex forms of *cis-trans* isomerism are possible. Natural oleic acid, thus, is a *cis*-form.

Simplified Structural Formulæ.—Many natural compounds, such as steroids and alkaloids, have very complex formulæ, and it is convenient to simplify the diagrammatic representation of their structure. This is done by extending the convention adopted in organic chemistry for benzene and other aromatic, or benzenoid, derivatives, in which the carbon atoms and their attached hydrogens, $-\text{CH}_2-$ and $-\text{CH}=\text{}$, are indicated by angles, the symbols for the elements being omitted.

\diagup represents $\begin{array}{c} \text{CH}_2 \\ \diagup \end{array}$, the saturated *ane*, or *methane* linkage.

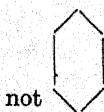
$\diagup \diagdown$ represents $\begin{array}{c} \text{CH} \\ \diagup \diagdown \end{array}$, the unsaturated *ene*, or *methene* linkage.

Where an unsaturated double or triple-linkage bond is not indicated, it is assumed that the bond C is carrying two H atoms, and the linkage is fully saturated. For this method of simplified representation to be unambiguous, special care must be taken to denote *every* unsaturated linkage in the diagram.

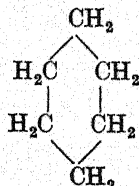
Thus, benzene and its derivatives should be represented :—



Benzene
 C_6H_6

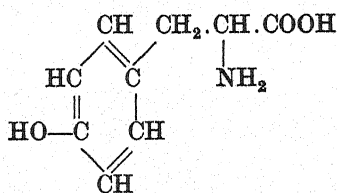


not $\diagup \diagdown$, which would denote

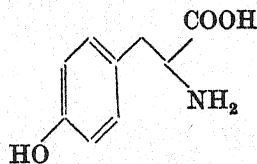


Hexahydrobenzene
 C_6H_{12}

Reactive groups and short side-chains, $-\text{OH}$, $-\text{CH}_3$, $-\text{NH}_2$, $=\text{CO}$, $-\text{COOH}$, and the like, are denoted as such. Long side-chains may be written in simplified form.

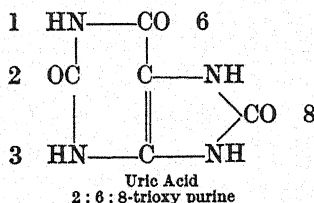


Tyrosine
p-Hydroxyphenyl alanine

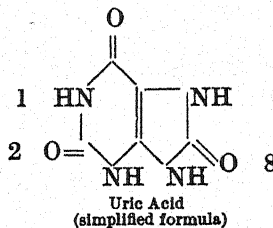


Tyrosine
(simplified formula)

The system may be applied to all types of organic compounds, including those in which N, O or another element forms part of a ring.



Uric Acid
2 : 6 : 8-trioxypurine



Uric Acid
(simplified formula)

The systematic nomenclature used in organic chemistry is gradually being adopted in biochemistry. According to this, the name of a compound is constructed to denote its chemical structure, type and characterising groups.

(1) The total number of C atoms is shown by a Greek numeral prefix :—

Pentane, a saturated hydrocarbon containing 5 carbon atoms.

Pentose, a sugar containing 5 carbon atoms.

(2) Saturation of all the carbon bonds is denoted by *ane*; unsaturation in any degree is shown by the vowel change of *a* to *e*, *ene*.

(3) The suffix shows either the general type of the compound or the presence of significant groups. Thus, the alcoholic groups, $-\text{CH}_2.\text{OH}$, $=\text{CH}.\text{OH}$, and $=\text{C}.\text{OH}$, are denoted by *-ol*, while *diol* or *triol* show the number of alcoholic hydroxyls present. The ketone group, $=\text{CO}$, is *-one*, and the amino group, $-\text{NH}_2$, is *-ine*, while the acidic carboxyl group, $-\text{COOH}$, is *ic* or *oic*.

REFERENCE

SMITH, C. (1936), "Modern Chemical Nomenclature." *J. Chem. Soc.*, 1067.

CHAPTER 6

THE CARBOHYDRATES, OR GLUCIDES

Definition.—Carbohydrates are neutral compounds composed of carbon, hydrogen and oxygen in which the H : O ratio is 2 : 1, as shown by the carbohydrate type formula $C_m(H_2O)_n$. Chemically, they are polyhydroxy alcohols with potentially active aldehyde or ketone groups. Analytically, they are all reducing sugars or give rise to reducing sugars on hydrolysis. Biologically, they represent one of the three principal forms in which carbon is transported, stored and utilised in living organisms.

Nomenclature.—Carbohydrates were originally classified as :

<i>Saccharides.</i>	<i>Polysaccharides.</i>
(The sugar group.)	(The starch-cellulose group.)
All soluble in water to form true solutions.	Higher members insoluble ; lower members form colloidal solutions in water.
Sweet taste.	No sweet taste.
Crystallisable.	Not crystallisable.
No colour reaction with iodine.	Higher members give blue or brown colour with iodine.
	All are resolved by hydrolysis into saccharides.

The name "carbohydrate" records the fact that in these compounds the H : O ratio is similar to that in water, H_2O . The name "glucide" indicates that all the members of the family are either sugars (or "glucoses") or compounds giving rise to one or more sugars on hydrolysis ("glucosides"). In modern nomenclature the name of each carbohydrate ends in the suffix "ose" ; the prefix indicating the number of C atoms in the molecule. Thus, *hexose* denotes a glucide, or carbohydrate, containing six C atoms.

For classification, carbohydrates are now divided into :—

- (A) *Simple sugars*, or *monosaccharides*.
- (B) *Compound sugars*, composed of two or more monosaccharides.
- (C) *Polysaccharides* formed by the condensation of several units of a monosaccharide.
- (D) *Heterosaccharides*, polysaccharides containing non-sugar residues.
- (E) *Glycosides*, compounds of a sugar and a non-sugar residue.
- (F) *Saccharide derivatives*, oxidation, reduction and condensation products obtained from and reconvertible into sugars.

All simple sugars contain a potentially active *aldehyde*, $-\text{CHO}$, or *ketone*, $-\text{CO}-$, group; and in consequence may be termed aldose or ketose sugars, according to the nature of the group.

Classification of the Carbohydrates.—A. Monosaccharides.—Simple sugars having the general formula $\text{C}_n\text{H}_{2n}\text{O}_n$. All are soluble in water, have a sweet taste, and reduce alkaline solutions of copper.

- (1) Monose, CH_2O , *Formaldehyde*. Theoretically the first member of the series, but not regarded as a sugar, owing to absence of the appropriate physiological characters.
- (2) Diose, $\text{C}_2\text{H}_4\text{O}_2$, *Glycolaldehyde*, $\text{CH}_2(\text{OH})\cdot\text{CHO}$.
- (3) Trioses, $\text{C}_3\text{H}_6\text{O}_3$. *Glycerose*, an aldotriose, the reference sugar in classification. *Dihydroxyacetone*, a ketotriose.
Trioses are the simplest *typical* sugars.
- (4) Tetrose, $\text{C}_4\text{H}_8\text{O}_4$. Four aldotetroses and a ketotetrose are known.
- (5) Pentose, $\text{C}_5\text{H}_{10}\text{O}_5$. Eight aldopentoses are known, including *arabinose*, *D-xylose*, and *D-ribose*. Also six methylpentoses, or methylsoses, have been found; their formula being $\text{C}_5\text{H}_9(\text{CH}_3)\text{O}_5$. *L-rhamnose* is the most familiar.
- (6) Hexose, $\text{C}_6\text{H}_{12}\text{O}_6$. Sixteen aldohexoses and eight ketohexoses are known. The chief aldohexoses are *D-mannose*, *D-glucose*, and *D-galactose*; the chief ketohexose is *D-fructose*.
- (7) Heptose, $\text{C}_7\text{H}_{14}\text{O}_7$. Five heptose sugars are known.
- (8) Octose, $\text{C}_8\text{H}_{16}\text{O}_8$. Three octose sugars are known.
- (9) Nonose, $\text{C}_9\text{H}_{18}\text{O}_9$. Two members are known.
- (10) Decose, $\text{C}_{10}\text{H}_{20}\text{O}_{10}$. One member is known.

B. Compound Saccharides.—Sugars formed by union of two or more monosaccharides, and convertible into monosaccharides by hydrolysis.

- (1) *Disaccharides*.—The chief disaccharides belong to the class of dihexosides, or sugars formed by union of two hexose molecules, with elimination of one water molecule. The general formula is $(\text{C}_6\text{H}_{11}\text{O}_5)-\text{O}-(\text{C}_6\text{H}_{11}\text{O}_5)$, or, briefly, $\text{C}_{12}\text{H}_{22}\text{O}_{11}$. Ten dihexosides are known, the most important being :—

Reducing Hexosides.	Non-reducing Hexosides.
<i>Maltose</i> = glucose-glucoside	<i>Sucrose</i> = fructose-glucoside
<i>Cellose</i> = glucose-glucoside	<i>Trehalose</i> = glucose-glucoside.
<i>Lactose</i> = glucose-galactoside.	

In the non-reducing sugars the aldehyde group participates in the linkage, and is not available for reducing the copper reagent.

- (2) *Trisaccharides*.—Sugars formed by condensation of three monosaccharides, with elimination of two water molecules. The general formula is $C_{18}H_{32}O_{16}$, examples being *raffinose* (galactose-galactose-glucose), and *melezitose* (glucose-glucose-fructose), both non-reducing sugars.
- (3) *Tetrasaccharides*, $C_{24}H_{42}O_{21}$.—*Stachyose* is the only example known.

✓ **C. Polysaccharides**.—Carbohydrates of high molecular weight and colloidal dimensions. Like compound saccharides, they are formed from monosaccharide units, but, unlike compound saccharides, they have no typical sugar characteristics, such as sweetness and true solubility.

Polysaccharides are classified according to the monosaccharides they liberate on hydrolysis.

- (1) *Pentosans*, $(C_5H_8O_4)_n$. Polymers of pentoses. Examples are *araban*, from gum arabic, and *xylan*, from straw.
- (2) *Hexosans*, $(C_6H_{10}O_5)_n$. Polymers of hexoses. Important examples are : dextrin, starch, cellulose, glycogen, and lichenin, all of which are *glucosans*, or polymers of glucose.
Inulin, a *fructosan*, or polymer of fructose. Mannan, a polymer of mannose.

D. Heterosaccharides.—Complex polysaccharides containing non-sugar residues in addition to saccharide units.

- (1) *Heteropentosans*. Gums, mucilages, pectic substances, hemicelluloses.
- (2) *Heterohexosans*. Lignocellulose, pectocellulose, lipocellulose, chitin.

E. Glycosides.—Compounds containing a saccharide and a non-sugar, or *aglucone*, residue. Many highly active drugs, such as digitalis and strophanthin, are glycosides.

When the saccharide residue is glucose the compound is often called a "glucoside," although in modern sugar chemistry this term is also applied to any compound saccharide containing glucose as one of its units.

F. Saccharide Derivatives.

- (1) Saccharide alcohols, or mannitols, formed by reduction of sugars.
- (2) Saccharide acids, formed by partial oxidation of sugars.
- (3) Saccharide esters and ethers.
- (4) Aminosaccharides, hexosamines.

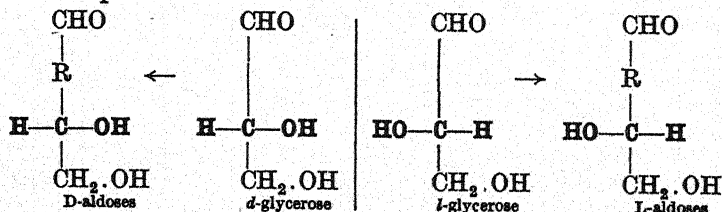
The Optical Properties of Carbohydrates.—All carbohydrates contain one or more asymmetric carbon atoms in which the carbon is united to four different kinds of components. This type of configuration endows the compound with (i) optical activity and (ii) optical or stereo-isomerism. By optical activity is meant the power possessed by the compound, both in crystalline form and in solution, of rotating in one or other direction a beam of plane-polarised light sent through it.

Optical activity is not directly observable in ordinary light, but requires the use of a polariscope which cuts out all the non-polarised light from the projected beam. To determine the direction and degree of rotation imparted to the beam, an analyser is fixed on the eye-piece of the polariscope, and can be turned in either direction until it compensates exactly for the rotation imparted to the light by the asymmetric compound. By this means the optical activity, expressed in terms of dextro-rotation (+) or lævo-rotation (−), to the clockwise right or anti-clockwise left of the observer, can be measured.

For every optically active dextro- or lævo-rotatory compound, a corresponding stereo-isomer, or epimer, exists, having the same formula but exactly opposite optical properties. This can only be explained by assuming that the groups attached to each asymmetric carbon atom can be arranged in either of two ways, and that the fact that chemical compounds occupy tri-dimensional space must be recognised in constructing formulæ.

When this is done, the structural formula of an optically active compound appears as the mirror-image of the formula of the corresponding epimeric compound.

All simple sugars can be regarded as derivatives of a primitive sugar, *glycerose*, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CHO}$, got by partial oxidation of glycerol, $\text{CH}_2\text{OH}.\text{CHOH}.\text{CH}_2\text{OH}$. Glycerose contains an asymmetric C atom, and exists in two isomeric forms: *dextro*-glycerose and *lævo*-glycerose. Sugars lineally derived from the *dextro*-glycerose are called *dextro* or D-sugars, in distinction to *lævo* or L-sugars, which are derived from *lævo*-glycerose. *Dextro*-glucose may be written D-glucose or *d*-glucose, the latter is usual, but the former is preferable.



Since glycerose has only one asymmetric carbon atom, C, only two optical isomers, or epimers, are known. In higher sugars the number of asymmetric carbons increases, and the number of epimers increases, in accordance with van't Hoff's rule: $n = 2^c$; where n is the number of possible isomers, and c the number of asymmetric carbon atoms present in the compound. Thus, a hexose with four asymmetric carbons, has sixteen isomeric forms.

The H—C—OH group furthest from the aldehyde or ketone group in the sugar is the reference group. All D-sugars have a terminal configuration in this respect similar to *d*-glycerose.

Much confusion has arisen from the indiscriminate use of the small prefix *d* or *l* to denote chemical relationship as well as optical rotation, and to avoid mistakes it is now usual to employ the prefix (+) or (−) to indicate if a compound is dextro- or lævo-rotatory, the large capital D or L being used to denote lineal descent from the (+) or (−) chemical ancestor.

Thus, D (−) aldopentose is the five-carbon lævo-rotatory sugar derived from *d* (+) glycerose; L (+) ascorbic acid is the vitamin derived from *l* (−) glycerose. It will be noticed that the direction of optical rotation of the derivatives is not necessarily the same as that of the parent compound.

The close physical and chemical similarity existing between these pairs of optical isomers renders them indistinguishable in many of their reactions, but in the region of molecular dimensions found in the living cell, and ruled by the enzymes, the distinction between stereo-isomers becomes of primary importance in determining their susceptibility to attack. Carbohydrates and proteins represent the two great families of organic biological compounds constructed of optically active units.

Naturally Occurring Sugars

MONOSACCHARIDES.

Sugar.	Sources.
(a) <i>Pentoses</i>	
$C_5H_{10}O_5$	
1. D-arabinose . . .	The glycoside <i>aloin</i> ; tubercle bacilli.
2. L-arabinose . . .	As the polymer <i>araban</i> in bran, husks, fruit skins; gum arabic, cherry and other gums; beet pulp; pectin;
3. D-ribose . . .	Nucleic acid, riboflavin.
4. D-xylose . . .	As the polymer <i>xylan</i> in straw, husks, canes; and in wood gums.
5. L-xyloketose . .	In pentosuric urines.

(A) MONOSACCHARIDES—*continued*.

Sugar.	Sources.
(b) <i>Hexoses</i> $C_6H_{12}O_6$	
6. D-glucose . . .	As free sugar in fruits, flowers, honey ; as a glycoside in compound saccharides ; as a glycoside in many plants ; as a polymer in cellulose, starch, dextrin and glycogen.
7. D-fructose . . .	As free sugar in fruits, flowers, honey ; as a glycoside in sucrose, raffinose and stachyose.
8. L-glucose . . .	In the glycoside <i>capsularin</i> .
9. D-galactose . . .	As a glycoside in compound saccharides, including lactose ; in glyco- or galactolipides ; polymerised as galactan.
10. L-galactose . . .	In flax seed mucilage.
11. D-mannose . . .	As glycoside in compound saccharides ; as a glycoside in plants ; in muco- and other proteins ; as the polymer <i>mannan</i> in ivory nut.

THE MONOSACCHARIDES

✓ **Pentoses.**— $C_5H_{10}O_5$.—These five-carbon sugars occur chiefly as pentosans and as glycosides. They are characteristic constituents of the nucleic acids of plants and animals, but never occur as free sugars, except in the pathological condition of **pentosuria**, when they are found in the blood and urine of animals.

The commonest pentoses are : *L-arabinose*, found in the arabans of gum arabic, cherry gum, and peach gum ; *D-xylose*, from the pentosans of straw, bran, and wood ; *D-ribose*, from nucleic acid, riboflavin (vitamin B_2), and co-enzymes I and II.

The nature of the pentose excreted in urine is variable and depends on diet as well as on disease.

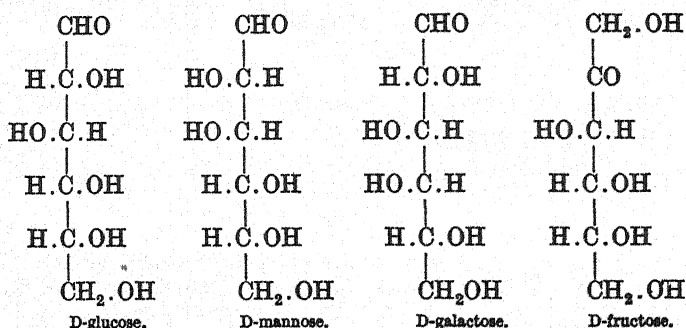
L-xyloketose is the commonest pentose found in pentosuria.

Pentoses may be obtained quantitatively by acid hydrolysis of the appropriate pentosan, and are detected by colour reactions (p. 126).

✓ **Hexoses.**— $C_6H_{12}O_6$.—Out of twenty-four possible hexoses, only four are capable of utilisation by higher animals. These are the aldohexoses, *glucose*, *mannose*, and *galactose*, and the ketohexose *fructose*. All are D-sugars, being related structurally to *D-glycerose*.

The inter-relationship of these four isomers is shown in the so-called *projection formulæ* on p. 92.

✓ **D-Glucose**, *grape sugar*, or *dextrose*, is widely distributed, both free and combined in glucosides, compound saccharides and polysaccharides. It occurs free in fruit and plant juices, in blood and



tissue fluids of animals, and in the alimentary tract as an end-product of carbohydrate digestion. Pure glucose is a white, crystalline solid. It melts at 100° C., losing one molecule of water of crystallisation at 110° C. Anhydrous glucose melts at 146° C. Further heating produces a brown mixture of decomposition products, termed *caramel*.

Glucose is very soluble in water, the solution being about one-third as sweet as sucrose of the same concentration. It is dextro-rotatory, $[\alpha]_D = +52.2^\circ$. Glucose is a typical aldose sugar in all its reactions. It reduces alkaline copper reagents, forms a characteristic osazone with phenylhydrazine, and is readily fermented by yeasts and bacteria.

D-Mannose is a relatively rare sugar, of slight importance in nutrition. It is obtained by acid hydrolysis of its natural polymer, *mannosan*, found most abundantly in Spruce sawdust and in the vegetable-ivory nut from the Tagua Palm. In concentrations of 0.5–4.0 per cent. mannose occurs in many higher proteins.

✓ **D-Galactose** is obtained from the hydrolysis of lactose, its chief source; from raffinose; from the galactans of gums and mucilages; and from the galactolipides, or cerebrosides, of the central nervous system. It resembles glucose, but is much less sweet, and less soluble in water. It is dextro-rotatory, $[\alpha]_D = +80^\circ$. On partial oxidation, it forms the sparingly soluble *mucic acid*, by which it may be identified.

✓ **D-Fructose**, *fruit sugar*, or *levulose*, accompanies glucose in fruits, flowers, and their product, honey. It is obtained also from the hydrolysis of fructosides, the chief of which is sucrose, and from the polysaccharide, inulin. It crystallises with difficulty in fine, colourless needles, m.p. 110° C. Fructose differs from the other three fermentable hexoses in being a keto-sugar, or ketose, and in being lævo-rotatory, $[\alpha]_D = -92.0^\circ$. It is much sweeter and more reactive than glucose.

THE STRUCTURE OF THE SIMPLE SUGARS

Taking glucose as an example of the most familiar aldohexose, its structure and properties can be expressed by at least a dozen different formulæ. Each of these represents a stage in the history of sugar chemistry.

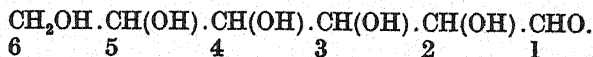
The Constitution of Glucose.—(1) $(\text{CH}_2\text{O})_n$, the *elementary formula*, found by combustion, shows that the substance has the C, H, and O, in the carbohydrate ratio.

(2) The *molecular formula*, $C_6H_{12}O_6$, calculated from the osmotic pressure, shows that the substance is of the hexose type. Mannose, galactose, and fructose have similar molecular formulæ.

(3) $C_6H_7(OH)_5O$. Acetylation by acetyl chloride, CH_3COCl , gives a penta-acetyl derivative, showing that the original sugar contained five $-OH$ groups. Mannose, galactose, and fructose have similar formulae.

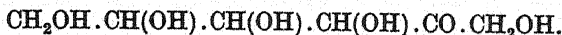
(4) The *aldose formula*, $C_5H_8(OH)_5.CHO$. Glucose contains one aldehyde, or $-CHO$, group, as shown by its oxidation to the corresponding carboxy acid, *gluconic acid*, $C_5H_8(OH)_5.COOH$, and also by its interaction with phenyl hydrazine to form a hydrazone. Mannose and galactose have similar aldose groups. Fructose does not contain an aldehyde group, and is represented by the *ketose formula*, $C_5H_7(OH)_5 : CO$, showing the ketone group : CO .

(5) The *linear formula*,



On complete reduction, glucose, mannose, and galactose are each converted into the same straight chain hydrocarbon, *hexane*, C_6H_{14} . This shows that the aldehyde group in the sugars must be terminal and not attached to some side-chain. Furthermore, the general stability of the sugars indicates that the five hydroxyl groups are attached to five different carbon atoms. Numbering to the left in accordance with chemical convention, the linear formula is 1-aldo-2 : 3 : 4 : 5 : 6-pentahydroxy-hexane. This formula applies equally well to glucose, mannose, and galactose.

The linear formula for fructose is :—



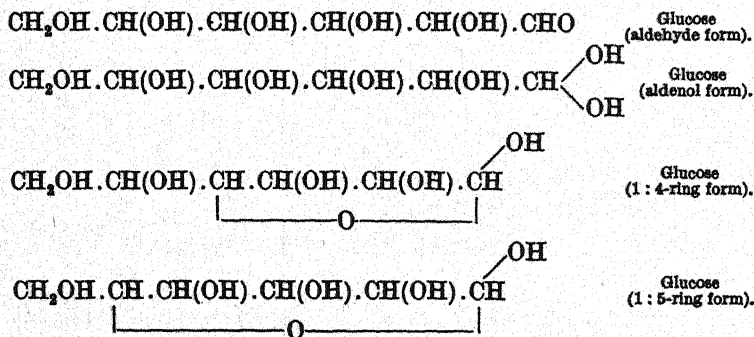
(6) *Projection Formulæ*.—The simple linear formula applies equally well to any of the aldohexoses, and to distinguish between these sugars the formula must be elaborated. This was done by Emil Fischer, who showed that the difference lay in the way in which the four secondary alcohol groups, —CH(OH)— , were turned. By altering their arrangement on either side of a vertical

line, sixteen different modifications become possible, some of which are shown on p. 92. Each modification corresponds to a different sugar, but the chief ones of biological importance are glucose, mannose, and galactose. Projection formulæ are of great use in classification of carbohydrate structure and in theoretical organic chemistry.

(7) *Cyclic Formulæ*.—The simple linear formula fails to explain some reactions of the sugars. For example, aldohexoses, while giving many of the reactions of aldehydes, do not produce a colour on addition of Schiff's aldehyde reagent. This indicates that the —CHO group must be masked or combined in some way. Furthermore, a fresh solution of hexose in water shows the phenomenon of *mutarotation*; the optical activity changes until it reaches an equilibrium value, which suggests that a mixture of sugars is formed. When a pentose, hexose, or any higher carbohydrate is distilled with excess of a strong acid the distillate always contains furfural derivatives.

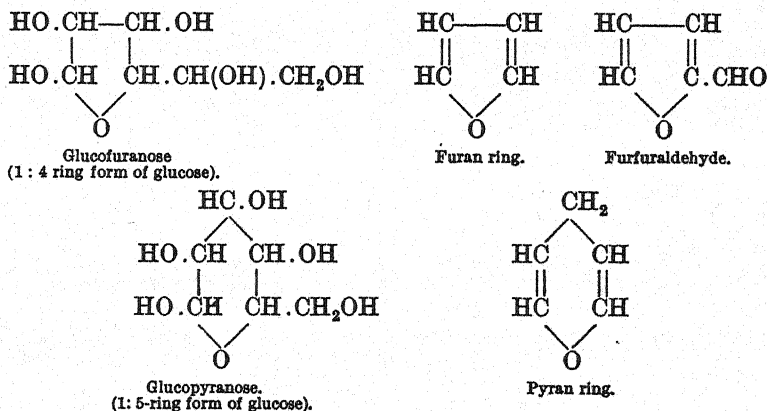
Furfural is a ring compound, and is latent in some way in the carbohydrate molecule. All these reactions can be explained by assuming that aldoses in solution undergo (a) hydration of the aldehyde group to an *aldenol* group, $\text{—CHO} + \text{H}_2\text{O} \rightarrow \text{—CH(OH)}_2$, and (b) subsequent condensation between one of the hydroxyls of the unstable aldenol group and one of the secondary alcohol groups to form a ring compound of a *lactone* type.

The identification of this secondary alcohol group presents difficulties. Evidence suggests that the fourth or fifth carbon atom, possibly both, may be involved, giving rise to two distinct cyclic forms of hexose.



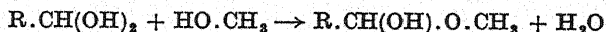
The 1:4-ring structure is called the *furanose* form, and the 1:5-ring structure is called the *pyranose* form of the sugar, to show that they may be regarded as derivatives of the respective cyclic

compounds, *furan* and *pyran*. The relationship is clearer when the sugars are written in cyclic form.

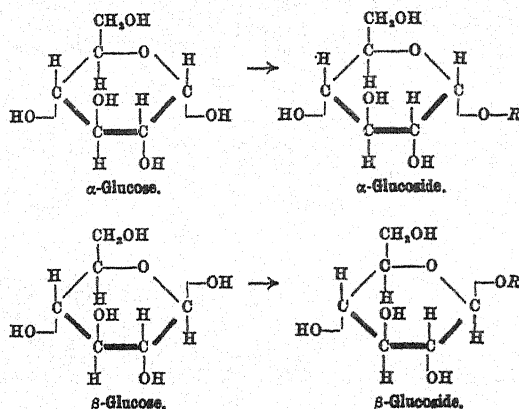


Furan is a 5-membered ring containing one oxygen atom, its aldehyde derivative, furfural, is obtained almost quantitatively when pentoses are distilled with strong acids. Higher sugars, such as hexoses, yield substituted furfurals under the same conditions. Pyran is a 6-membered ring containing one oxygen atom. The relative importance of these formulæ is disputed. Haworth concludes that glucose occurs chiefly as the pyranose form. The furanose sugar, being much more unstable, tends to revert to the pyranose form.

α - and β -glucose.—When glucose and other sugars are dissolved in methyl alcohol, and treated with HCl gas, monomethyl glycosides are obtained which no longer have the power of reducing alkaline copper solutions, showing that the aldenol group has been united to the methyl alcohol.



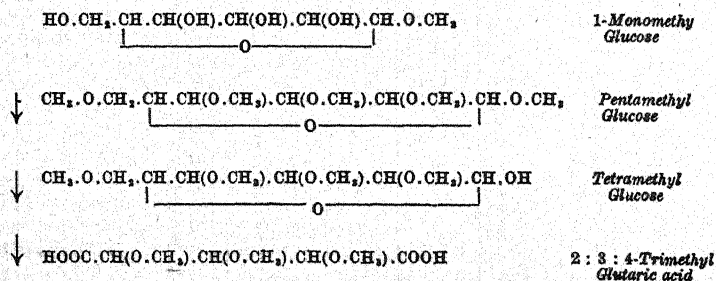
Each sugar, however, is able to yield two different glycosides, showing that some difference must exist between the two hydroxyls of the aldenol group. By careful hydrolysis, Fischer was able to recover two forms of glucose from these ethers: α -glucose, of which the rotatory power is $+113^\circ$, and β -glucose, of which the rotatory power is $+19^\circ$. Ordinary glucose when freshly dissolved in water decreases in rotatory power until it reaches an equilibrium value of $+52.2^\circ$, showing that it is now a mixture of both α and β forms. The existence of these sugars is due to the fact that the terminal carbon 1, which is symmetric when in the form of the $-\text{CHO}$ or the $-\text{CH}(\text{OH})_2$ group, becomes asymmetrical directly one of the hydroxyls has condensed with a carbon 5 hydroxyl to produce the pyranose ring. The phenomenon of *mutarotation*, or change in rotatory power, and the existence of α - and β -glycosides is further proof that sugars normally exist in a ring structure. This is indicated in the modern *perspective* formulæ of the carbohydrates in which the plane of the ring is represented as being at right-angles to the plane of the paper, the various H and OH radicles being attached to perpendiculars.



In these perspective formulæ, attached H and OH are represented as projecting either above or below the plane of the ring. This allows for the existence of the sixteen isomeric aldohexoses required by Fischer's linear formulæ.

Evidence for the 1:5-oxygen Linkage in Glucopyranose.—The Raman spectra of sugars in solution does not show the shift in region of 1,600–1,700 wave number, characteristic of the carbonyl group, $=CO$, which indicates that aldehyde or ketone groups do not occur free in the sugar molecule. All true sugars have at least one terminal primary alcohol group, $-CH_2.OH$, and at least one secondary alcohol group, $-CH(OH)-$.

When a methyl glucoside is treated with methyl sulphate it is completely methylated, all the available $-OH$ being converted into methoxy groups, $-O.CH_3$. Subsequent hydrolysis preferentially removes the original methyl glucoside group, leaving a tetra-methyl glucose. This on oxidation with HNO_3 forms a trimethyl derivative of glutaric acid, showing that the oxygen bridge in the original sugar must have been between the carbons 1 and 5.



THE COMPOUND SACCHARIDES

Compound saccharides are formed by condensation of two or more monosaccharides. Thus, a disaccharide contains two monosaccharides, a trisaccharide contains three, and a tetrasaccharide contains four.

(B) COMPOUND SACCHARIDES, OR OLIGOSACCHARIDES

Sugar.	Sources.	Components.
(a) <i>Dihexosides</i> — $C_{12}H_{22}O_{11}$ Maltose (malt sugar) Dextrinose (isomaltose) Lactose (milk sugar) Cellobiose Gentiobiose Melibiose Turanose Sucrose (saccharose, cane sugar) Trehalose	Starch hydrolysis Glycogen hydrolysis Beer, honey, liver Milk of all mammals Cellulose hydrolysis The glycoside amygdalin; the trisaccharide gentianose The trisaccharide raffinose The trisaccharide melizitose Fruits and sugar-storing plants, cane and beet In fungi; as a polymer tre- halum in ergot and moulds	glucose + glucose glucose + glucose galactose + glucose glucose + glucose glucose + glucose galactose + glucose glucose + fructose glucose + fructose glucose + glucose
(b) <i>Trisaccharides</i> $C_{18}H_{32}O_{16}$ Raffinose Gentianose Melezitose	Sugar beet, cotton seeds, molasses Gentian root In manna of Douglas fir, honeydew	galactose + glucose + fructose glucose + glucose + fructose glucose + glucose + fructose
(c) <i>Tetrasaccharide</i> $C_{24}H_{44}O_{21}$ Stachyose	In leguminous seeds	glucose + glucose + galactose + fructose

Disaccharides.—Theoretically, any pair of monosaccharides may unite to form a disaccharide, but the term is commonly restricted to the *dihexose saccharides*, $C_{12}H_{22}O_{11}$, since they include the most familiar natural sugars, sucrose and lactose.

The general properties of a disaccharide depend on (i.) the constituent saccharides, and (ii.) the manner of linking. If the aldehyde or ketone groups are unaffected by the mode of union, the disaccharide is a reducing sugar, and, like all the monosaccharides, is able to react with alkaline copper solutions, such as those of Fehling and of Benedict. If, however, the aldehyde or the ketone groups of the constituent monosaccharides are immobilised by the

linkage, the resulting disaccharide is a non-reducing sugar. This distinction is of considerable analytical importance.

A. Reducing Disaccharides (potentially active groups present).

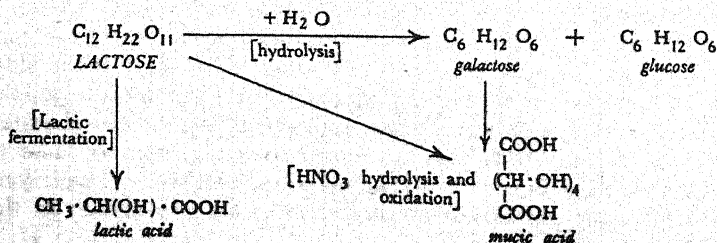
✓ **MALTOSE**, or *malt sugar*, a glucose-glucoside produced during the hydrolysis of starch and glycogen by acids or enzymes. It is the characteristic sugar of *malt*, or germinating barley, being formed by the breakdown of the reserve starch. It is very soluble in water, and is dextro-rotatory, $[\alpha]_D = +137.5^\circ$. The sugar is a typical aldose and gives a characteristic osazone. Maltose is easily hydrolysed by acids or the enzyme *maltase* into two molecules of glucose.

Since maltose is not attacked by the enzymes sucrase, lactase, or emulsin, all of which hydrolyse β -glucosides, it is inferred that it is an α -glucoside of α -glucose.

✓ **LACTOSE**, or *milk sugar*, is found in the milk of all mammals. Its percentage ranges from about 2 in the rabbit, to about 7.5 in the elephant. Human milk has a lactose range of 5–7 per cent. Cow's milk contains 3.5–4.8 per cent. The sugar appears in the blood and the urine during lactation. It has not been detected conclusively in plants or in lower animals. Lactose is much less soluble and less sweet than the other common sugars, and is not fermented by ordinary yeasts. It is dextro-rotatory, $[\alpha]_D = +52.5^\circ$, has the properties of an aldose, and gives a characteristic osazone. Oxidation with nitric acid converts it, *via* galactose, into mucic acid, a sparingly soluble crystalline compound. This reaction serves to distinguish lactose, galactose, and their derivatives from all other sugars.

Lactose readily undergoes lactic fermentation by many organisms found infecting milk, notably *Streptococcus lactis*. This leads to the formation of lactic acid, and the consequent "souring" of the liquid. Lactose is hydrolysed by a specific enzyme, *lactase*, found in the mammalian intestine, in emulsin (from almonds), and in some yeasts. It is not attacked by maltase, sucrase, or diastase, and is somewhat more resistant to acid hydrolysis than maltose or sucrose. The products of hydrolysis are the monosaccharides, glucose and galactose, showing that it is a galactoside, actually, 4- β -galactosido-glucose.

Reactions of Lactose.—



CELLOBIOSE, or cellulose, a β -glucose-glucoside obtained indirectly when the polysaccharide *cellulose* is acetylated and hydrolysed; the yield shows that at least one-half of cellulose is composed of cellobiose units. Cellobiose is hydrolysed by acids, by emulsin, lactase, and the specific enzyme, *cellobiase*, but not by maltase. The end-products are two molecules of glucose. In its properties cellobiose resembles maltose, but is less sweet and less soluble. It is not attacked by maltase, and hence is assumed to be a β -glucoside.

Maltose and cellobiose have now been shown to be stereo-isomers. Maltose is 4- α -glucosido-glucopyranose and cellobiose is 4- β -glucosido-glucopyranose.

Cellobiose has not been found to occur free in nature.

GENTIOBIOSE is found condensed with fructose in the trisaccharide *gentianose* from Gentian root. It is 6- β -glucosido-glucose. Gentiobiose is used in bacteriology to distinguish between closely related organisms, such as *Bact. coli*, which does not attack it, and *Aerobacter aerogenes*, which does.

MELIBIOSE, a glucose- α -galactoside found condensed with fructose in the trisaccharide *raffinose* from beetroot. It is hydrolysed to glucose and galactose by acids, and by the enzyme *melibiase*, found in lower fermentation yeasts but not by "top-fermentation" yeasts. Consequently, the sugar is used to distinguish between these two yeast types. Melibiose is 6- α -galactosido-glucose.

B. Non-reducing disaccharides.

SUCROSE, *saccharose*, *cane sugar*, *beet sugar*, the most important industrial sugar, is widely distributed in plants, notably the stems of the sugar-cane and sugar millet, the root of the sugar-beet, and the trunks of some palms and maples. Sucrose crystallises readily as large, colourless monoclinic crystals, m.p. 160° C., which are soluble in water to the extent of 67 gm. per 100 gm. of solution at 20° C. The sugar is non-reducing, and does not show mutarotation in solution, which indicates the absence of potentially active aldehyde or ketone groups. It is readily hydrolysed by dilute acids, forming a molecule of glucose and one of fructose. Chemically, sucrose is an α -glucoside of fructose. Sucrose is dextro-rotatory, $[\alpha]_D = +66.5^\circ$, but after hydrolysis the resulting mixture of monosaccharides is lævo-rotatory (owing to the liberation of fructose), and this mixture is termed "invert sugar." Consequently, hydrolysis of sucrose is often described as *inversion*. The enzyme capable of causing it is called *invertase*, the systematic name being *sucrase*, which shows the particular sugar it attacks.

TREHALOSE, found in fungi and seaweeds, resembles sucrose generally, but is not hydrolysed by sucrase. By the action of

acids or the enzyme *trehalase*, which accompanies it in fungi, it is converted into two molecules of glucose.

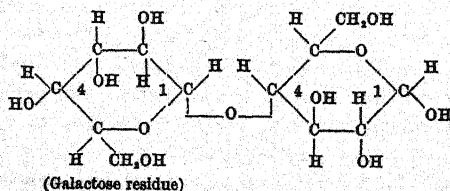
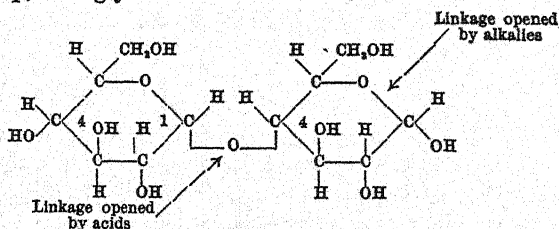
Trisaccharides.—Compound sugars formed by union of three monosaccharides. The most important are the *trihexosides*, $C_{18}H_{32}O_{16}$, of which four are known. Only one of them, mannotriose, is a reducing sugar.

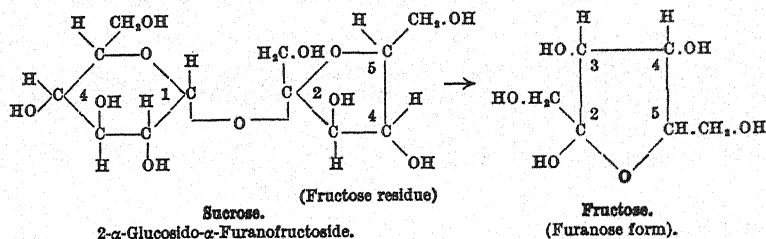
RAFFINOSE, the commonest trisaccharide, occurs in beetroot, cotton-seed (which contains 8 per cent.), and to a less extent in cereals and fungi. It is non-reducing, is dextro-rotatory, and closely resembles sucrose, m.p. $118.5^{\circ}C$. Structurally, raffinose is a fructo-gluco-galactoside, and liberates a molecule of each of these sugars on complete hydrolysis.

Trisaccharides have been detected in mammalian blood and tissue fluids, where they contribute to the "bound-sugar" fraction that only shows reducing properties after hydrolysis.

Tetrasaccharides.—A non-reducing tetrahexoside, stachyose, $C_{24}H_{42}O_{21}$, occurs in the seeds of leguminous plants. It is derived from glucose, fructose, and two molecules of galactose. On partial hydrolysis, glucose is split off, leaving the trisaccharide mannotriose.

The Structure of the Compound Saccharides.—The simplest formula for a disaccharide is $(C_6H_{11}O_5)-O-(C_6H_{11}O_5)$, showing the union of the two monosaccharide residues by an oxygen linkage, which is the point of hydrolytic attack. In reducing disaccharides one of the residues, A, retains its aldehyde or ketonic properties as a reducing group. B, the other residue, by means of its own reducing group, is in glycoside union with A.





The glucose component in sucrose is in the α form, the fructose component is liberated on hydrolysis as fructofuranose. Hence the structure ascribed to sucrose is 2- α -glucosido- α -furanofructoside.

POLYHEXOSIDES, OR POLYSACCHARIDES

These carbohydrates differ profoundly from the sugars. Of high molecular weight, they do not form true solutions in water, but are either colloidal or insoluble. They have no sweet taste. They give none of the sugar reactions characteristic of aldose or ketose grouping.

Instead, many of them react chromatically with iodine, a property not possessed by the sugars. The carbohydrate nature of the polysaccharides is shown by their conformity to the type formula, $C_m(H_2O)_n$, and by their cleavage into simple sugars on complete hydrolysis. Biologically, polysaccharides constitute the food reserve (starch), and structural material (cellulose, lignose, pentosan) of plants, and the easily mobilised carbohydrate reserve (glycogen) of animals. Substituted polysaccharides (heterosaccharides) are found as structural carbohydrate in the chitin of crustaceans and insects, and the pectin of fruits. All are classified chemically as *Holosaccharides* and *Heterosaccharides*.

HOLOSACCHARIDES.—Polysaccharides composed entirely of sugar units.

- (1) **Pentosans.**— $(C_5H_8O_4)_n$, polymers of a pentose unit. Chiefly *arabans* (from arabinose) and *xylans* (from xylose). Pentoses occur mostly in combination with other materials in vegetable gums and mucilages.
- (2) **Hexosans.**— $(C_6H_{10}O_5)_n$, polymers of a hexose unit. The most important members are the *glucosans*, or polymers of glucose, including cellulose, starch, glycogen, and their derivatives.

HEXOSANS

CELLULOSE, one of the chief organic products of vegetation. The cell-wall of all young plants is made of cellulose; in older cells

other materials are incorporated, forming hemi-cellulose, ligno-cellulose, pecto-cellulose, and adipo-cellulose.

Industrial Sources of Cellulose

Cotton, flax	90-75 per cent. pure cellulose. Used for fine textiles.
Hemp, ramie, jute, manilla.	75-55 per cent. cellulose. Used for coarse textiles, wrapping, and cordage.
Wood-pulp, cereal straw, esparto.	40-20 per cent. cellulose. Used for paper-making and many other purposes.

Cellulose is obtained in quantity from cotton-wool. After being cleaned by alkaline washing, the product, either as filter-paper or cotton, is nearly pure cellulose. Low-grade paper is made from ligno-cellulose, which becomes friable and yellow on exposure to air and light.

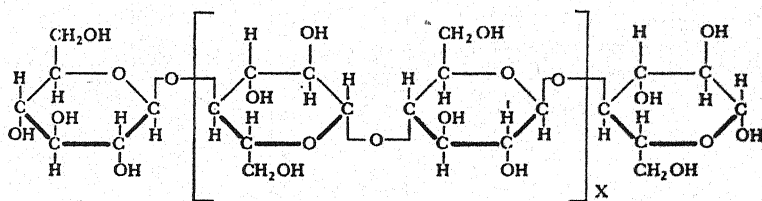
Pure cellulose is a white, somewhat hygroscopic material, insoluble in water and common organic solvents, and relatively inert towards chemical reagents. It may be dissolved in : (a) ammoniacal cupric hydroxide (Schweitzer's reagent), (b) zinc chloride in hydrochloric acid, (c) acetic anhydride, (d) sodium hydroxide and carbon disulphide, (e) concentrated sulphuric acid. In these reagents cellulose undergoes various changes giving rise to important industrial products, artificial silks, and plastics, such as "viscose," "cellophane." When dissolved in a mixture of nitric and sulphuric acid, cellulose forms the basis of gun-cotton, celluloid, collodion, and some types of artificial silk.

Cellulose is not acted on by the alimentary enzymes of higher animals, but is attacked by the widely distributed cytases, or cellulases found in germinating cereals, fungi, moulds, and bacteria.

The abundant cellulose in the diet of herbivora is digested by the flora found in the paunch and cæcum of ruminants, and the soluble carbohydrates liberated are absorbed by the host. Cellulose is not attacked in the human intestine. It is credited with the property of promoting peristalsis by mechanical stimulation, and forms part of the insoluble residue or "roughage" of the dietary.

On hydrolysis, cellulose yields glucose exclusively, and when acetylated and hydrolysed simultaneously (acetolysis) it yields 50 per cent. of cellobiose (4- β -glucosido-glucose), which indicates that cellulose is a chain of β -glucosidic units.

According to Haworth and his colleagues, cellulose has a molecular weight of the order of 30,000, and contains 100-200 glucose units.



Cellulose

M. Wt., 20,000-40,000.

Number of C_6 units, 100-200. $X = 80-100$.

STARCH, or *Amylum*.—Starch is present in quantity in most vegetable foodstuffs, in cereals, and their products such as bread. It is characterised by giving a deep blue colour with dilute iodine.

Starch does not occur in animal tissues. It has been synthesised by Hanes (1940), from glucose-1-phosphate.

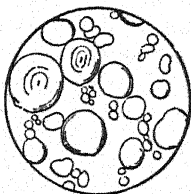
Starch is found in solid granules throughout plant tissues in leaves, stems, roots, fruits, and seeds. It is absent from a number of monocotyledons, notably the Snowdrop, Iris, and Hyacinth. The granules are usually made up of concentric layers formed round a hilum, suggesting that the material is deposited during cycles of activity. When viewed by polarised light, a black cross is seen branching from the hilum.

The shape and size of the granule is characteristic of the plant, being ovoid and irregular in potato and arrowroot, oval in beans, discoid in wheat and barley, and polyhedral in rice and maize.

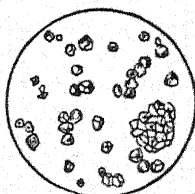
The starch granule is an aggregation of starch molecules which are resolved and rendered water-soluble by mild acid or enzyme hydrolysis, yielding "soluble starch."



Potato.



Wheat.



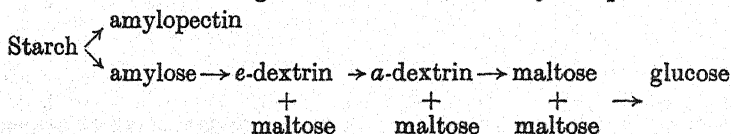
Rice.

FIG. 3.—Starch Grains ($\times 200$).

Average diameters in μ of common starches are: potato, 140-180; arrowroot, 155; haricot bean, 65; pea, 45-55; wheat, 45-55; maize, 30; oat (composite granules), 35-45; oat (single granules), 6-8; rice (single granules), 4-6.

Amylose and Amylopectin.—In 1858, Nägeli recognised two separate constituents of the starch granule, an external "starch cellulose" and an internal material, "granulose," the precursor of maltose. A separation was effected in 1906 by Maquenne and Roux, who termed the inner material "amylose," and obtained an 80 per cent. yield. "Starch cellulose" they renamed "amylopectin." Amylose gives a blue iodine reaction, is soluble in water, and is completely hydrolysed to maltose by malt amylase. Amylopectin is insoluble in water, gelatinises on being boiled, and causes the gel formation in starch paste. Its colour reaction with iodine is variable, as different forms of amylopectin exist. Amylopectin is a phospho-carbohydrate, and before it is hydrolysed it must be dephosphatized. This may be accomplished by barley phosphatase. Subsequent treatment with malt diastase (amylase) converts the carbohydrate into a mixture of maltose, isomaltose, and glucose.

Products of Starch Hydrolysis.—Amylose is much more easily hydrolysed by acids and enzymes than cellulose. The process is both erosion and cleavage of the molecule, and may be represented:—



Saccharification is never complete. A residue of amylopectin, phosphates, and hemi-cellulose remains, its composition depending on the nature of the starch. Hydrolysis may be traced by means of the iodine test or by sugar estimation.

Waldschmidt-Leitz has obtained from malt two enzymes capable of hydrolysing starch in two different ways, as shown by the time at which the iodine blue colour is no longer given. α -amylase is a dextrinogen, and attacks the starch in the centrally placed glucoside linkages, producing dextrins. Under the attack of α -amylase, the blue reaction ceases to be given when about one-tenth of the starch has been converted into maltose, the rest being now a mixture of dextrins. Conversely, β -amylase is a saccharogen, and splits off maltose, unit by unit, from the ends of the starch molecule. Under its attack, the blue reaction persists until nearly all the starch is turned into maltose, the formation of dextrins being almost completely suppressed.

Dextrins are intermediate products of hydrolysis of starch and glycogen. They differ from starch in being soluble in cold water, and from sugars in being more readily precipitated by alcohol. The higher members, *e*-dextrins (erythro-dextrins), give red colours with iodine, the lower members, *\alpha*-dextrins (achroo-dextrins), give

no colour with iodine. All have a high dextro-rotation in solution, hence the group name, and all are completely hydrolysable to sugar. Purified by alcohol precipitation, they appear as white or yellow amorphous powders, readily soluble in water. The copper-reducing power depends on the method of preparation, and non-reducing dextrins have been obtained. Dextrins do not occur free in nature. Their presence imparts a characteristic flavour to bread crust, toast, and partly charred cereal foodstuffs.

DEXTRIN, or Starch Gum.—Industrial dextrin is made by heating dry potato starch to 210° C., or by autoclaving starch paste in presence of 1 per cent. citric acid. The product is chiefly α -dextrin, and is an important adhesive.

Maltodextrin is the general term applied to non-crystalline copper-reducing intermediate products of the action of diastase on starch.

GLYCOGEN, or animal starch, is found principally in liver and muscle, and also in other animal tissues, in all foetal tissues, in many fungi, and in maize seed. It is a rapidly mobilised carbohydrate, and is an essential constituent of the muscular machine. Glycogen closely resembles a higher dextrin. It is a white, water-soluble powder, $[\alpha]_D = +196.6^{\circ}$. It gives a mahogany red with iodine, and is precipitated from aqueous solution by addition of alcohol up to 60 per cent., or by saturation with solid ammonium sulphate. It does not reduce alkaline copper solutions. Hydrolysis by acids, diastase or amylase, converts it first into lower dextrins, then maltose, and finally glucose. Glycogen may be distinguished from α -dextrin by its opalescence in solution, and by its precipitation by basic lead acetate. It has been synthesised by Cori, from glucose-1-phosphate.

Glycogen is relatively stable in hot 30 per cent. KOH or NaOH, and may be extracted by these reagents from fresh, finely minced tissues. It is purified by alcoholic reprecipitations, and by dialysis.

Glycogen Distribution in Animals.—Mammalian liver contains 3–7 per cent. of glycogen, or about a quarter to a half of the total reserve of the animal. The remainder is chiefly in muscle, which contains up to 1 per cent. Starvation rapidly lowers the liver glycogen value, but affects the muscle glycogen much less.

Structure of the Polyhexosides.—The molecular weights of these compounds is very high, and different values are obtained by different methods, which suggests that the carbohydrate is affected by the analytical treatment.

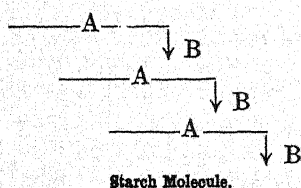
Thus, molecular weight determinations of acetylated starch give a minimal value of $(C_6H_{10}O_5)_{18}$ for the starch molecule, but this may have been lowered by depolymerisation during acetylation.

As regards composition, the pure polyhexosides are composed entirely of hexose units, most, if not all, of which are present in disaccharide form, as cellulose in cellulose, and as maltose in starch and glycogen. These units are linked together to form long chains, the chains themselves possibly being united laterally by secondary attractions between neighbouring groups. Glycogen is a typical spherical colloid, cellulose is a linear colloid, and starch is intermediate in type.

The fact that the polysaccharides as a class have no sweet taste suggests that the secondary alcohol groups, —CH.OH , that confer sweetness and solubility on organic compounds, have been combined in some way during polymerisation of the sugar units. This polymerisation is different from condensation, such as occurs in the formation of ethers and esters, since polysaccharides, with the exception of lignose and cellulose, are easily depolymerised by dilute acids.

Although the individual chain seems to be made up of some 10–30 glucose units, in aqueous solution the molecules aggregate to form polydispersed particles, or macro-molecules, which have molecular weights ranging from 60,000 to 300,000, the average being 200,000. From a study of the X-ray diagram of the crystal lattice of a typical polysaccharide (cellulose), Sponsler and Dore support the conclusion that the molecule is a chain of sugar units, each in pyranose form, united by β -linkages.

According to Haworth (1939), the typical starch molecule is an aggregate of repeating units, each of which is an unbranched chain of 24 to 30 glucose residues, the chains being arranged in a laminated pattern held together at intervals by glycoside linkage.



A is the chain of glucose residues united by 1 : 4- α -pyranose linkage.

B is the glycoside cross-linkage, formed by condensation of a terminal $\text{—CH}_2\text{.OH}$, or —CH(OH)— , with a corresponding group in the next chain.

Evidence for this is based on identification of the products of the hydrolysis of fully methylated starch. These include 2 : 3 : 6-trimethyl-glucose, derived from the units of the unbranched chains, which, therefore, must be cross-linked by the C1 and C4 groups, respectively. Estimation of the yield of 2 : 3 : 4 : 6-tetramethyl glucose provides a means of measuring the number of free end-groups of each chain in the polysaccharide.

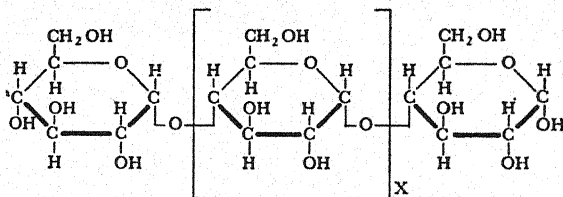
According to Freudenberg (1939), at least four different polysaccharide types exist :—

(1) Open, unbranched chains of sugar units, as in cellulose, and possible chitin and pectin.

(2) Open, branched chains, as in starch and glycogen.

(3) Five-membered rings with branches, as in levulan.

(4) Five-membered, unbranched rings, as in the crystallisable dextrins. Sedimentation rates, as found by the ultra-centrifuge, show that glycogen and starches have high molecular weights and can exist in various degrees of aggregation. X-ray analysis shows that polysaccharide chains, such as cellulose, are not kinked like those of the protein of wool or silk, which explains the lower degree of stretching shown by cotton and similar carbohydrate fabric material.



Starch and Glycogen Chain

Starch : A mixture of homologues of M. W. 10^4 to 10^6

Glycogen : M. W. 10^6 to 4×10^6

INULIN, or Dahlia starch, is the commonest example of a **fructosan**, or polymer of fructose. It is a reserve carbohydrate found in the roots and leaves of Compositæ and other plants. The chief sources are the tuber of the Dahlia, which contains 10 per cent., the Jerusalem Artichoke, Chicory, and Dandelion. It is not found in animals. Inulin is a white, tasteless powder, closely resembling starch, but giving no colour with iodine. Its solutions are lævoptatory, $[\alpha]_D = -40^\circ$, non-reducing, and not hydrolysable by amylases. When acted on by acids or the enzyme *inulase*, which accompanies it in the plant, inulin is converted completely into fructose.

Inulin forms a trimethyl derivative which yields trimethylfructofuranose on hydrolysis, showing that the polysaccharide is a chain of fructofuranose units. Irvine and Haworth believe the molecular weight to be about 5,000. Inulin is effectively hydrolysed and absorbed in the human intestine, possibly with the aid of the inulase-secreting micro-organisms.

Levan is a synthetic polysaccharide of fructose formed by the action of *B. mesentericus* or *B. subtilis* on sucrose. It is structurally different from inulin in the linkage of the fructose units.

Levosin occurs in wheat flour, rye, bran and stubble. On hydrolysis it yields glucose and fructose in the proportions of 1 : 9.

Holosaccharides

Name.	Class.	Formula.	Chief Source.
Araban . . .	Pentosan.	$(C_5H_8O_4)_n$	Gum arabic.
Xylan . . .	"	"	Straw, bran.
Cellulose . . .	Glucosan.	$(C_6H_{10}O_5)_n$	Plant fibre.
Starch . . .	"	"	Cereals, tubers.
Dextrin . . .	"	"	Starch.
Glycogen . . .	"	"	Liver, muscle.
Inulin . . .	Fructosan.	"	Dahlia tubers.
Levan . . .	"	"	Synthetic.
Levosin . . .	Gluco-fructosan.	"	Cereals, bran.

HETEROSACCHARIDES. Polysaccharides containing carbohydrate and non-carbohydrate units. They are classified according to the sugar residues yielded on hydrolysis :

- (1) **Heteropentosans.**—Mucilages, gums, pectic substances, hemi-celluloses, all of which contain pentose units.
- (2) **Heterohexosans.**—Lignocellulose, pectocellulose, lipocellulose, all of which contain hexose units.
- (3) **Mucopolysaccharides.**—Hexosamine derivatives present in mucins and mucoids.

Pectins, or vegetable mucilages, are widely distributed in plant tissues, roots, fruit pulp and rind, including the peel of apples, oranges and lemons. They are not extracted by cold water, but when boiled with weak acids, such as occur in fruits, or when attacked by the enzyme *pectase*, they are hydrolysed into substances which form characteristic jellies on cooling.

A typical pectin, such as that found in beetroot, yields (i) a polysaccharide, araban, and (ii) a complex acid, pectic acid, which is present as a calcium magnesium salt in the original pectin, and causes the gel formation.

Agar-agar, a mucilage from certain red seaweeds (*Rhodophyceae*), contains 20–30 per cent. of galactan, a polymer of galactose, combined as an organic sulphate. Agar gels are superior to gelatin gels in bacteriological work. They do not melt at as low a temperature, and they are not attacked by proteoclastic micro-organisms.

Lignocellulose.—As plant tissues age, the cellulose of the stems becomes lignified, or converted into lignocellulose, the final form

of which is wood. The change is due to combination between the cellulose and lignin (or lignon), which is a pentosan united to an aromatic residue, such as vanillin, *m*-methoxy-*p*-hydroxybenzaldehyde, $C_6H_3(OH)(OCH_3).CHO$.

Mucopolysaccharides

Natural mucins and mucoid secretions contain polysaccharides, either free or united to proteins as glycoproteins, and thus constitute a field common both to carbohydrate and protein chemistry. The polysaccharides are characterised by the presence of amino-sugar, or *hexosamine*, residues, and are classified by Meyer (1938) as (i) neutral mucopolysaccharides, (ii) mucopolysaccharides containing uronic acid, and (iii) mucopolysaccharides containing both uronic and sulphuric acid residues.

Neutral mucopolysaccharides of known composition are represented by (i) chitin; (ii) gastric polysaccharide, from gastric mucosa; and (iii) bacterial polysaccharides.

Uronic sulphate-free mucopolysaccharides occur in vitreous humour, umbilical cord, synovial fluid, and certain bacteria.

Uronic sulphate-containing mucopolysaccharides are represented by *chondroitin sulphate*, in cartilage and other connective tissues; *mucoitin sulphate*, from gastric mucin and from cornea; *heparin*, an anti-coagulant present in liver and lung tissue.

Chitin forms the basis of the exo-skeleton of the *Insecta* and the *Crustacea*, and enters into the supporting tissue of fungi. It is a polymer of acetyl glucosamine, $CH_3.CO.NH.C_6H_{11}O_5$, with at least four units, and is the only prominent example of the use of a carbohydrate as skeletal material in heterotrophic organisms. Chitin is non-reducing and gives no colour with iodine. It is an insoluble and very stable carbohydrate, but may be hydrolysed by acids to an equimolecular mixture of glucosamine and acetic acid.

SACCHARIDE DERIVATIVES

(1) **Saccharide Alcohols, or Mannitols.**—Every simple sugar may be regarded as derived from a parent hydroxy alcohol, which can be obtained by reduction of the aldehyde or the ketone group of the sugar.

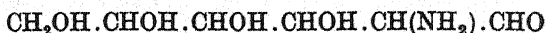
At least a dozen of these carbohydrate alcohols have been found widely distributed in plants, where they may represent a form of carbohydrate storage or a by-product in sugar metabolism. The most familiar are: *Erythrol*, $C_4H_8(OH)_4$, found in algae and mosses; *D-Mannitol*, $C_6H_{14}(OH)_6$, the alcohol corresponding to mannose, found widely distributed in fungi and manna from tree sap, especially that of the larch; *D-Sorbitol*, an isomer of *D-Mannitol*, found in the fruit of the mountain ash and most *Rosaceae*.

L-Sorbitol is used in one of the laboratory methods for synthesising the vitamin Ascorbic acid (p. 290). *Glycerol*, the trihydroxy alcohol corresponding to glycerose, is a characteristic constituent of oils and fats found in animals and plants.

Chemically, the saccharide alcohols are stable, non-reducing substances, soluble in water and in alcohol, and possessing a very sweet taste. They are not fermented by yeasts, but are attacked by various moulds and bacteria, and are used in bacteriological work as a means of distinguishing between different organisms.

(2) **Saccharide Acids.**—These are formed by the oxidation of a terminal group in a monosaccharide. *D-glucuronic acid* is the most familiar, and is found as a detoxicant ester in urine after phenol administration. It occurs also in mucoproteins and a few hetero-saccharides. Its formula is given on p. 118. *L-Galacturonic acid* occurs in pectic acids.

(3) **Amino Saccharides.**—Sugars in which an OH group has been replaced by an amino group, $-NH_2$. The natural amino saccharides are *hexosamines* of the general formula :



D-Glucosamine, or Chitosamine, obtained quantitatively by the hydrolysis of the polysaccharide *Chitin*, and as a final product of the hydrolysis of mucoitic acid from mucoproteins (p. 143). It is 2-amino glucose.

Chondrosamine, 2-amino galactose, is obtained by hydrolysis of chondroitic acid, a component of cartilage.

(4) **Saccharide Ethers.**—Saccharides may form various ethers, or compounds connected by an oxygen linkage, $-C-O-C-$. When these ethers are made up of two or more sugar molecules the product is a true sugar and belongs to the class of compound saccharides. When the ether is formed by the union of a sugar and a non-sugar or *aglucone* organic partner the product is a *glycoside*.

Glycosides comprise an important group of plant constituents, including the anthocyanin pigments that colour flower petals, the tannins, and many drugs, such as digitalis and strophanthin. Animal glycosides are represented by the cerebrosides and the nucleosides.

Esters are formed by union of saccharides with various acids, the hexose phosphates being of special interest since their formation precedes decomposition of the sugar molecule in metabolism.

THE HEXOSE PHOSPHATES

Hexose Diphosphate.—Fructofuranose-1 : 6-diphosphoric acid, (Harden-Young ester), $C_6H_{10}O_4(H_2PO_4)_2$, is formed in the first stage of the fermentation of glucose, mannose or fructose by yeast. The

phosphate on hydrolysis by boiling yields fructose, and its formation is an example of the interconvertibility of the three monosaccharides. It is also an early product in the conversion of glycogen into lactic acid, in muscle.

Fructose Monophosphate.—This ester has been obtained by Neuberg from the 1:6-diphosphate by partial hydrolysis. Unlike the diphosphate, it is fermented by yeast. Neuberg's ester is one of the two hexose phosphates found in muscle.

Glucose Monophosphates.—A crude monophosphate (Robison's ester) has been obtained by the action of yeast juice on glucose or fructose. It is a mixture of fructose monophosphate (Neuberg's ester) and glucopyranose-6-phosphoric acid (Embden's ester). The two esters have been separated by fractional crystallisation of their brucine salts. A glucopyranose-1-monophosphate (Cori's ester) is obtained by the action of phosphate on minced muscle.

Pentose phosphates occur naturally in the nucleic acids, each of which is made up of four *mononucleotides*, or glycosides of pentose monophosphate (p. 142). 5-phosphoribose occurs in the nucleic acid of animal chromatin, and in the inosinic and adenylic acid of muscle (p. 333). 3-phosphoribose occurs in the guanylic acid, xanthylic acid and nucleic acid found in yeast (p. 406).

All these esters are variously described as sugar phosphates or phospho-sugars. In each of them only one of the three hydroxyl groups of phosphoric acid has been bound, and they are acidic compounds. The process by which they are formed is termed *phosphorylation* (p. 239).

Dialysed extracts of muscle, liver, brain and yeast contain an enzyme that phosphorylates each glucose unit in glycogen and thus disrupts the polysaccharide into glucose-1-phosphate.

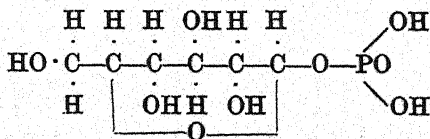


The reaction requires adenylic acid as a phosphate-carrier. Another enzyme in the extract catalyses the change:



the change being accelerated by Mg^{++} or Mn^{++} .

These transformations are of primary importance in carbohydrate metabolism.

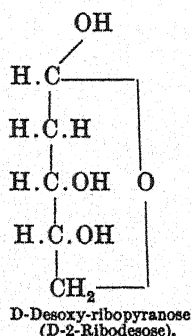
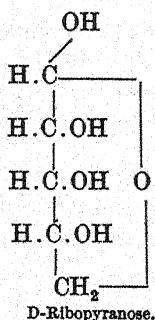
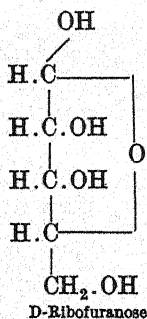


Glucose-1-phosphate (Cori ester).

The 1-monophosphate, unlike the 6-phosphate, is non-reducing, since the potential aldehyde group has been immobilised and cannot undergo dienolisation.

(5) **Glycals and Desoxy-sugars.**—By reduction and hydrolysis of substituted sugars it is possible to obtain unsaturated sugars, or *glycals*, in which two adjacent hydroxyl groups have been removed, leaving the linkage —CH=CH— . Glycals in presence of acid take up one molecule of water, thus restoring one of the hydroxyl groups, $\text{—CH(OH)—CH}_2\text{—}$, and forming a desoxy-sugar, which has one oxygen atom less than the parent monosaccharide. One of these sugars, 2-desoxy-D-ribose, occurs naturally in animal nucleic acid, while several ω -desoxy sugars (methyl sugars), in which the ω -, or terminal $\text{—CH}_2\text{.OH}$ group has become —CH_3 , are widely distributed among plants.

Examples of ω -desoxy sugars are: 3-desoxyglyceric aldehyde, or lactic aldehyde, $\text{CH}_3\text{.CH(OH).CHO}$, found in poplar leaves; 6-desoxy-D-galactose, found as a polymer in Japanese seaweed and in gum tragacanth; 6-desoxy-D-allose, or digitalose, found in the glycoside digitalin; and 6-desoxy-L-mannose, or L-rhamnose, found in many glycosides.



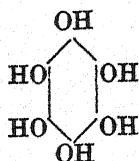
D-ribofuranose occurs in many important ribosides, notably riboflavin (vitamin B_2), adenylic, guanylic and inosinic acid, the co-enzymes I and II, and co-carboxylase, and in the nucleic acid from yeast.

The desoxy-ribose found in animal nucleic acid is derived from the pyranose form of the ribose, and, since the oxygen has been removed from carbon 2, is termed D-2-*ribodesose*.

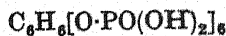
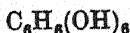
CYCLITOLS or CYCLOHEXANOLS

These compounds are isomeric with the saccharides, since they have the general formula $C_m(H_2O)_n$; they are, however, chemically

distinct from the sugars, being hydroxy derivatives of benzene in which there is no potential aldehyde or ketone group. On account of their hydroxylation, cyclitols are soluble in water and have a sweet taste. As a class they are stable, non-reducing compounds, and do not give the general tests for carbohydrates.



Inositol.

Phytic Acid
Inositol hexaphosphoric Acid.

Inositol, $C_6H_6(OH)_6$, hexahydroxy-hexahydrobenzene, is a cell constituent of almost all plant and animal tissues, being concentrated most in leaves, citrus fruits, cereal grain, yeast, fungi, bacteria, muscle, liver and the central nervous system. It is very soluble in water and has a sweet taste, which led to its original and inaccurate designation as "muscle sugar." Eight *cis-trans* isomers of inositol are possible, only one of which is optically inactive, the naturally occurring *i*-Inositol.

Inositol is a vitamin necessary for the growth of some yeasts, and for the maintenance of health and prevention of alopecia, or baldness, in rodents. Combined with six molecules of phosphoric acid, it forms *phytic acid*, the Ca and Mg salts of which are sparingly soluble, and are concentrated in the outer coat of cereals and other seeds, and provide a reserve of these metals for the growing plant. Since neither phytic acid nor CaMg phytate, or "*phytin*," is attacked by the enzymes of the human alimentary tract, they are useless in human nutrition, and free phytic acid, when in excess, is harmful, as it tends to combine with the available calcium of the diet, and may even "decalcify" the animal by promoting transference of Ca from the skeletal tissues to the alimentary tract.

The phytic acid content of cereals has been reviewed by Common (1940). The total phosphorus, expressed as percentage of dry matter is: wheat, 0.4; oatmeal, 0.35; bran, 0.65 to 1.5; beans, 0.66; grass, 0.35; yeast, 1.54.

The phytic acid content, expressed as percentage of the total P is: wheat, 65.7; oatmeal, 68.3; bran, 75 to 77; beans, 75.5; grass, 2.0; yeast, 3.8. From this it will be seen that phytic acid, free or as phytate, can account for about two-thirds to three-quarters of the total phosphorus of important food cereals.

CHAPTER 7

REACTIONS OF CARBOHYDRATES

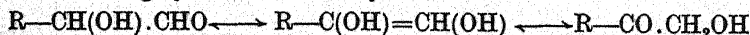
NATURAL carbohydrates are stable compounds under conditions of neutrality. Changes of reaction in the direction of acidity or alkalinity untabilise them in various ways, depending on the class to which they belong. The changes undergone include :—

- (1) Enolisation, interconversion and decomposition by alkalies.
- (2) Decomposition by strong acids.
- (3) Hydrolysis by weak acids.
- (4) Reduction.
- (5) Oxidation.
- (6) Hydrazone and osazone formation.
- (7) Esterification, and phosphorylation.

(1) **Decomposition by Alkalies.**—Polysaccharides and non-reducing saccharides (sucrose) are relatively stable in presence of alkalies, but reducing sugars are very unstable, and readily undergo three types of change : (a) non-oxidative molecular rearrangement, (b) non-oxidative molecular fission, and (c) oxidation and fragmentation. The type of the change depends on temperature, concentration of alkali, and presence of oxidising agents. Changes of the first and second type have been studied by Lobry de Bruyn and by Nef, and are extremely complicated. Changes of the third type occur when sugars are aerated in alkaline solution, the chief products being simple organic acids (lactic, pyruvic, propionic, acetic, glyoxylic, oxalic, and, finally, carbonic acid).

Heated in alkaline solution along with an oxidising agent, such as a cupric, ferric, or mercuric salt, all monosaccharides and all compound saccharides containing aldehyde groups are rapidly oxidised to acids containing a fewer number of carbon atoms. At the same time, the metal is reduced in proportion to the amount of the sugar present, and this reaction is made the basis of the principal methods for detecting and estimating reducing carbohydrates.

Interconversion of Sugars.—In alkaline solutions, even at room temperature, the oxygen bridge in reducing sugars is opened by spontaneous hydrolysis. This destroys the ring-structure, and exposes the aldehyde or ketone grouping, which rearranges by accepting a hydrogen atom from the adjacent alcohol group, and forms the highly reactive *dienol* system :—

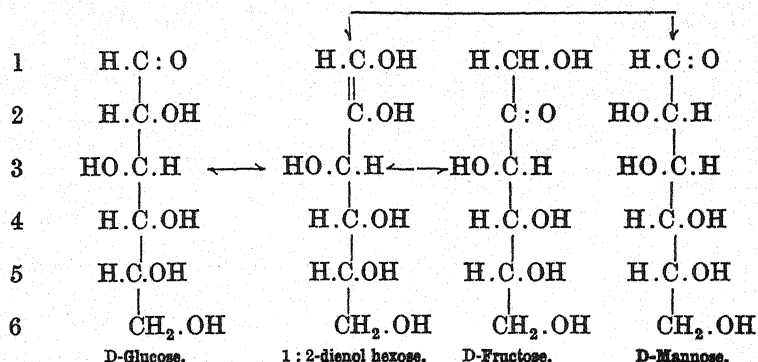


Aldose.

1 : 2-dienol sugar.

Ketose.

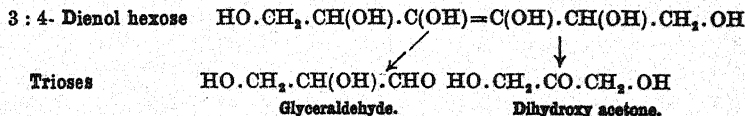
These three sugars exist in a state of equilibrium, and all three will be present when any aldose or ketose is kept in alkaline solution. The three hexoses, glucose, fructose and mannose, all yield the same 1 : 2-dienol, since the residual part of the molecule is the same in each sugar, and by the process of dienolisation the individuality of the original 1 and 2 carbon pattern is lost. Hence, in alkaline solution, any one of these hexoses gives rise to an equilibrium mixture that includes the other two sugars and the dienol common to all three.



A similar process of interconversion occurs in the organism, and all three hexoses are capable of being fermented by yeast, and used by the animal to form glycogen.

The dienol is a powerful reducing agent, and gives a violet colour with *o*-dinitro benzene in cold, alkaline solutions, while it also reduces ferric, mercuric and cupric ions, as may be seen in the familiar tests for reducing sugars.

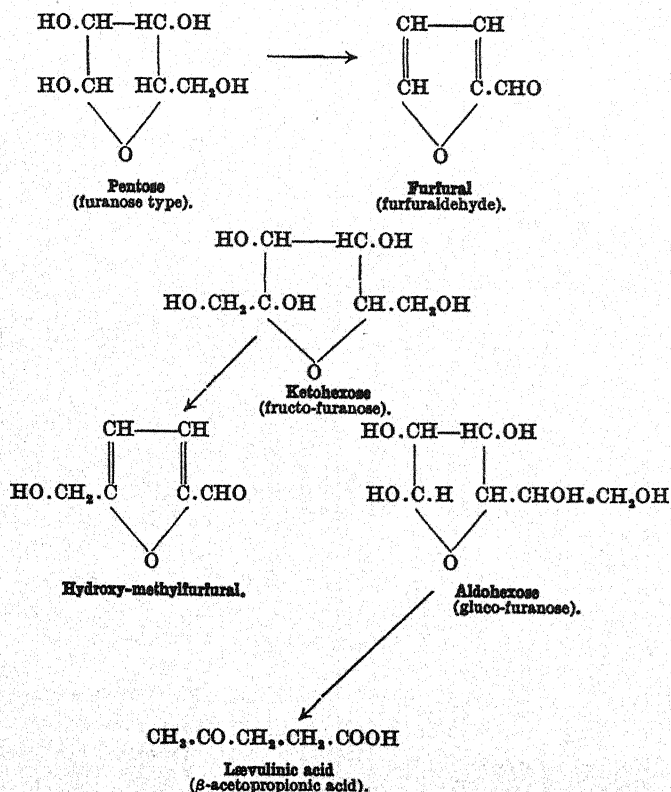
In more strongly alkaline solution, when protected from oxidation, the dienol double-bond may be transferred to the linkage between carbons 3 and 4, with subsequent cleavage of the hexose into two 3-carbon sugars, or trioses.



These trioses are believed to be the primary decomposition products of glucose breakdown, or glycolysis, in muscle and other tissues, the fission being effected by preliminary formation of phosphoric esters.

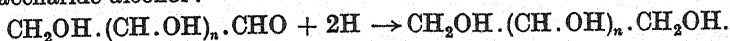
(2) **Decomposition by Strong Acids.**—All carbohydrates higher than tetroses on being boiled with excess of strong acids (HCl or H_3PO_4 is best) evolve *furfurals*, which may be detected by colour tests and separated by distillation. Furfural liberation is important as indication of a furanose pattern latent in the original carbohydrate. The type of furfural depends on the sugar residue present. Pentoses are quantitatively converted into simple furfuraldehyde on distillation with 20 per cent. HCl. Ketohexoses yield about 20 per cent. substituted furfural; and aldohexoses liberated about 1 per cent. of hydroxy furfural. These differences in reaction are used to distinguish pentoses, ketohexoses, and aldohexoses. Polysaccharides react in accordance with the nature of the saccharide units they contain.

Levulinic acid is the chief product of the action of concentrated acids on hexoses and hexosides. It may be regarded as arising by the opening up of the furan ring.



(3) **Hydrolysis by Weak Acids.**—Compound saccharides and many polysaccharides (starch, glycogen, inulin) are readily hydrolysed to simple saccharides by boiling with weak acids. The simple saccharides themselves are stable in weak acid solutions unless oxidising agents are present, when they are converted into organic acids, although much less rapidly than when they are in alkaline solutions.

(4) **Reduction.**—Reduction of an aldose produces the parent saccharide alcohol :—

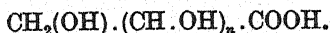


Reduction of a ketose produces a mixture of two isomeric hydroxy alcohols.

Saccharide alcohols closely resemble the original sugars in solubility, sweetness, optical activity, and isomerism. Once formed, they are very resistant to further reduction, and require drastic reagents, such as boiling HI, to reduce them to the parent hydrocarbon.

(5) **Oxidation.**—Aldoses may undergo oxidation in three different ways to produce acids having the same number of C atoms as the parent sugar.

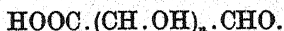
(i) Oxidation of the aldehyde group alone. This forms the corresponding *saccharinic acid* :



(ii) Oxidation of the terminal alcohol group as well as the aldehyde group. This produces the corresponding dicarboxy, or *saccharic, acid* :

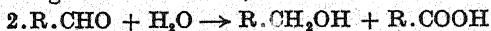


(iii) Oxidation of the terminal alcohol group alone. This can only occur if the aldehyde group is protected during the oxidation. The resulting compound is a *uronic acid* :



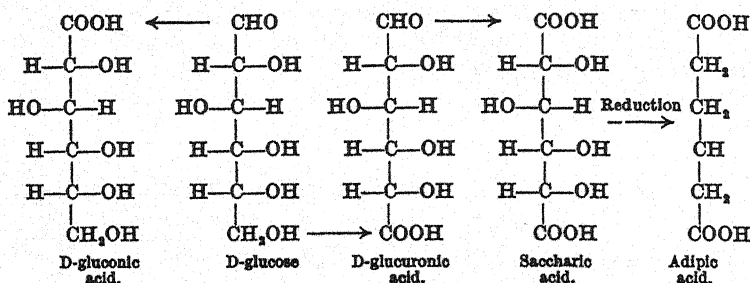
For example, D-glucose on oxidation with bromine water forms the saccharinic acid, D-gluconic acid. On oxidation with nitric acid, D-glucose forms saccharic acid. To obtain the aldehyde-acid, glucuronic (or glycuronic) acid, the glucose must be oxidised in the form of a glucoside, in which the aldehyde group is combined, and so escapes oxidation.

The Cannizzaro reaction.—In alkaline solution, aldehydes tend to undergo an oxidation-reduction transformation with production of the corresponding alcohol and acid, which is neutralised by the alkali.



In concentrated alkali, and in the absence of an oxidiser, aldehyde sugars by this transformation may give rise to saccharinic acids.

Saccharide Acids



D-glucuronic acid is the most important of the uronic acids. It is excreted in the urine as a glucoside after administration of higher alcohols, phenols, terpenes, chloral, and similar compounds. This represents a form of *detoxication* in which the foreign substance is rendered inert by being condensed with the aldehyde group of glucose. The resulting glucoside is partially oxidised to form the uronic compound.

Saccharic acid, tetrahydroxy adipic acid, forms a sparingly soluble acid potassium salt that is useful in identifying glucose and glucosides.

Mucic acid, an isomer of saccharic acid, is formed by oxidation of galactose and galactosides by means of warm nitric acid. It is almost insoluble in cold water, and provides an important means of identifying galactose.

The ketonic sugars are resistant to bromine oxidation, and thus may be separated from the aldoses. On more powerful oxidation, ketoses rupture at the carbonyl group, with formation of two acids.

Oxidation of compound saccharides is usually accompanied by hydrolysis unless special precautions are taken. Polysaccharides and non-reducing saccharides are relatively resistant to oxidation.

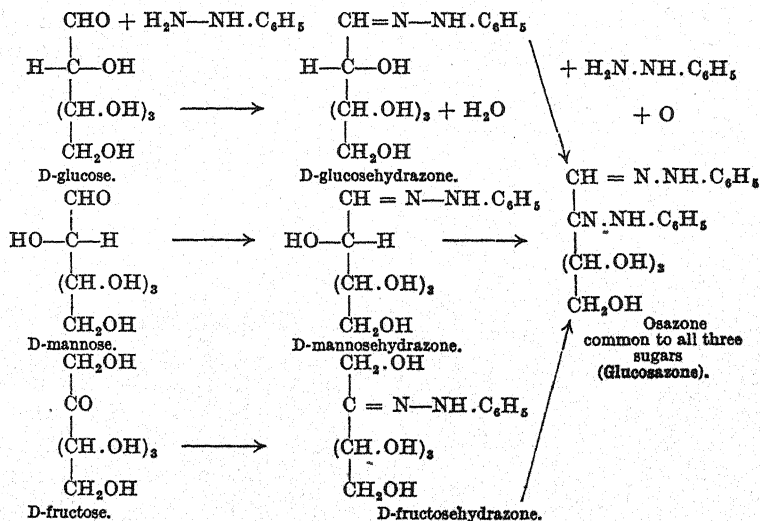
A reducing disaccharide on oxidation yields a monocarboxy *bionic* acid, the —CHO becoming —COOH. These acids, like all hydroxy acids with hydroxyl in the 3, 4, 5, or 6 position, readily close up to form a *lactone*, or ring compound made by condensation between —COOH and one of the —OH groups of the same carbon chain.

(6) **Hydrazone and Osazone Formation.**—Aldoses and ketoses react with hydrazines, or compounds of the type $R.NH-NH_2$. This involves replacement of the aldehydic or ketonic : O by the group $R.NH-N=$, the resulting product being called a hydrazone. Thus, by means of the reagent **phenylhydrazine**, $C_6H_5.NH-NH_2$, phenylhydrazones can be obtained from all the reducing sugars. These hydrazones, with the exception of mannose phenylhydrazone,

are freely soluble in water, and all can be reconverted into the sugar by treatment with benzaldehyde.

However, when the sugar phenylhydrazones are boiled with excess of phenylhydrazine an important reaction occurs. The alcohol group adjacent to the hydrazone radicle is oxidised to a ketone or an aldehyde group, which in turn reacts with phenylhydrazine to produce a double hydrazone, or **osazone**. Sugar osazones are sparingly soluble in water, have characteristic crystalline shapes and melting points, and provide a valuable means for sugar identification, alone or in mixtures.

Furthermore, D-glucose, D-mannose, and D-fructose yield the same osazone, showing that their molecules contain a similar arrangement of carbon atoms once the individuality of the C_1 and C_2 groupings has been lost by union with the hydrazine.



(7) **Esterification.**—Sugars can unite by means of their hydroxyl groups with acids to give a variety of esters, some of which occur naturally in the organism, while others have been of use in determining the constitution of the carbohydrates.

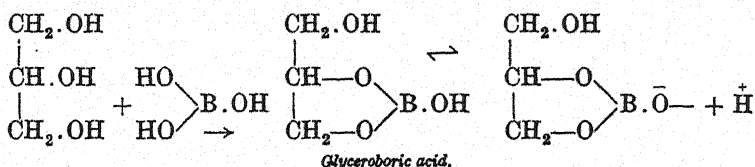
The formation of penta-acetates is evidence that hexoses only contain five free hydroxyl groups, the sixth oxygen being present in cyclic linkage. The discovery of two different methyl glucosides led in turn to the discovery of the α - and β -sugars.

With acetone, sugars give crystalline mono- and di-acetone derivatives, formed by union of the acetone: CO group with two adjacent —OH groups which must be in the *cis* position, or on the same side of the

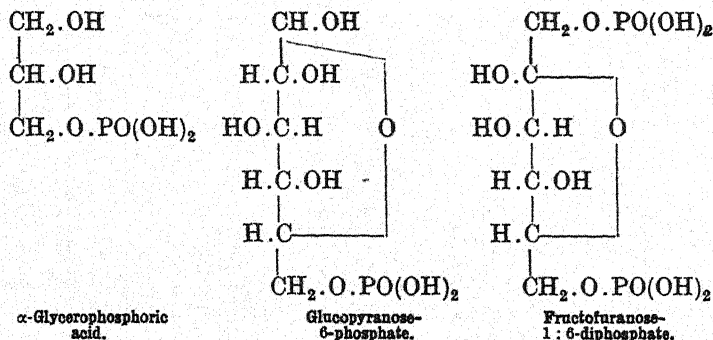
—C—C— axis. Since in these ethers two adjacent carbons are held in the crab-like grip of the —O—C—O— group they are termed *chelate* compounds. By previous formation of these acetone derivatives, pairs of *cis* hydroxyl groups in a sugar may be masked or protected during subsequent chemical manipulations, as, for example, those employed in the synthesis of ascorbic acid (vitamin C) from L-sorbose.

Sugars, glycerol and other polyhydroxy compounds also unite with the hydroxyl groups of weak acids to form esters that have the characteristic of being more acidic than the parent acid.

For example, boric acid, H_3BO_3 , is a very weak acid, and occurs in neutral solution largely as non-ionised $(\text{HO})_3\text{B}$. On addition of boric acid or a neutral borate to glycerol, esterification rapidly takes place at ordinary temperatures to produce a monoborate, which now displays acid properties and may be estimated by alkaline titration.



Phosphoric acid, H_3PO_4 , which occurs in solution as the ion $(\text{HO})_2\text{PO}.\text{O}-$, combines by means of one of its unionised hydroxyls to form glycerophosphoric and hexose phosphoric esters, which are of great biological importance.



(8) **Rotation of Polarised Light.**—By use of the polarimeter, all carbohydrates can be divided into a dextro-rotatory class and a laevo-rotatory class. The sugar D-fructose is the commonest laevo-rotatory saccharide, and for this reason is termed *levulose*. Each pure saccharide has a specific rotatory constant, which is determined under equilibrium conditions so as to avoid confusion due to mutarotation.

Specific Rotation.—This is defined as the rotation in angular degrees produced by a length of 1 decimetre of solution containing 1 gm. of solute in 1 ml. It may be expressed: $[\alpha] = \frac{100a}{lc}$, in

which $[\alpha]$ = specific rotation ;

a = observed angular rotation ;

l = length of the column of solution, in decimetres ;

c = concentration, in grams per 100 ml. of solution.

The value of the specific rotation depends on the temperature, which is fixed for reference purposes at 20° C. It also depends on the nature of the light source employed. The bright yellow D lines of the sodium spectrum or the yellow-green mercury line, 5461Å, are the usual illuminants. The light used is indicated by affixing D or Hg to the symbol denoting the specific rotation.

Sugar in Aqueous Solution.	$[\alpha]_D$	Sugar in Aqueous Solution.	$[\alpha]_D$
L-xylose . . .	+ 19.0	D-fructose . . .	— 92.0
D-arabinose . . .	—105.0	Lactose . . .	+ 52.5
L-rhamnose . . .	+ 8.9	Maltose . . .	+137.5
D-glucose . . .	+ 52.2	Sucrose . . .	+ 66.5
D-galactose . . .	+ 80.5	Invert sugar . . .	— 20.6
D-mannose . . .	+ 14.6	Raffinose . . .	+105.2

The technique of polarimetry depends very much on the types of instrument in use, details of which will be found in the books by Brown and Zerban (1941), Cole, Hawk and Bergeim, and Plimmer. Improvements and modifications in optical saccharimetry are described in "Recent Advances in Analytical Chemistry," Part II., edited by Ainsworth Mitchell.

ANALYTICAL REACTIONS OF CARBOHYDRATES

(1) General Test for Carbohydrates. The Thymol Test

To a small quantity of the solid or the solution (0.5 ml.) add 3 drops of 3 per cent. thymol in alcohol, 5 ml. of strong HCl, and about 2 gm. of solid NaCl, or enough to fill 1 cm. of the test tube. Boil *carefully* over a small flame, for 1–3 minutes.

A carmine colour is given by carbohydrates.

The NaCl is added to make the mixture boil quietly.

(a) The test may be applied equally well to insoluble carbohydrates, such as cellulose or wood, as the process of boiling with the acid brings about their solution.

(b) The pigment is due to condensation between furfural liberated from the carbohydrate and the thymol. It may be extracted by amyl alcohol, or by chloroform.

(c) Many other phenols may be used instead of thymol, such as α -naphthol (Molisch's original form of the test), resorcinol, etc.

(d) Higher proteins give the reaction owing to the presence of sugar residues (p. 147).

(e) The reaction cannot be applied to the detection of sugar in urine because the indoxyl present forms a pigment of its own with the phenol.

(f) Oxidation is necessary for the development of the colour.

(2) Group Test for Polysaccharides. Iodine Test

Mix a drop of 1 per cent. iodine with 5 ml. of water. Add the unknown solution drop by drop, and note any colour change.

<i>Deep blue.</i>	<i>Red-brown.</i>	<i>No change, other than pale yellow due to iodine.</i>
Starch.	Glycogen.	Polysaccharides absent.
	Dextrin.	

Neither inulin, a polysaccharide of fructose, nor achroo-dextrin gives a colour with iodine. The first is detected by hydrolysis, when it only liberates fructose (distinction from the non-reducing sugar, sucrose), the second is rarely met with free from erythro-dextrin.

The colour is due to an iodine adsorption complex of variable composition (Barger, 1930). It is discharged on warming or on addition of alkali, but returns on cooling or acidification.

(3) Group Test for Saccharides. Nitro-chromic Reaction

To 3 ml. of the solution add about 5 ml. of concentrated nitric acid and 5 drops of 5 per cent. potassium chromate. Mix well. A blue colour develops in about a minute if sugar be present.

(a) The test depends on the presence of $-\text{CH}.\text{OH}$ groups, and therefore is given by all primary and secondary alcohols, including glycerol, and also by formaldehyde, lactic acid, hydroxy-butyric acid, and mandelic acid.

(b) Polysaccharides free from sugars give no colour with the test until they have been hydrolysed by the action of the nitric acid. This may require some time, if the mixture is kept cool.

(c) The test is negative with proteins, fats, and the normal constituents of urine.

(4) Dienol Reactions

(1) **Alkali Test.**—To 5 ml. of solution add 10 drops of 20 per cent. sodium or potassium hydroxide. Warm gently. All the

reducing sugars show a colour change in the solution, passing from pale yellow to dark brown.

(a) This simple reaction, known as Moore's test, is due to dienolisation of the sugar, and subsequent "resinification" of the dienol. It is of not much analytical value, but serves as a basis for the "reduction tests."

(b) Polysaccharides and non-reducing sugars do not react.

(2) **Dinitrobenzene Reaction.**—Make 4 ml. of sugar solution alkaline with 5–10 drops of 20 per cent. sodium hydroxide. Add about 4 drops of 5 per cent. alcoholic *o*-dinitrobenzene, $C_6H_4(NO_2)_2$. Mix well, and warm the solution gently. A violet colour develops if a reducing sugar be present. If the reaction be positive, repeat the test, but do not heat the mixture. A violet colour developing in 15–30 minutes when the tube is kept at room temperature suggests that the sugar is fructose. Aldose sugars may require twice as long, as they are less rapidly enolised. Shaking the mixture with air inhibits the test by oxidising the reactants.

(a) *Ascorbic acid*, a typical dienol, gives an immediate violet with the cold alkaline reagent, but differs from the sugars in not giving a thymol test.

(b) Polysaccharides and non-reducing sugars do not react.

(c) Uric acid and creatinine both react on heating, so the test is not suitable for the detection of sugar in urine.

(3) **Methylamine Test for Reducing Disaccharides.**—Add 3–5 drops of 5 per cent. aqueous methylamine hydrochloride, $CH_3.NH_2.HCl$, to 5 ml. of sugar solution, which should be neutral. Boil vigorously. Remove from flame, and make strongly alkaline with 5–7 drops of 20 per cent. sodium hydroxide. Do not shake the mixture, as aeration inhibits the test. A yellow colour develops (dienol reaction), which changes to bright carmine if **maltose**, **lactose**, **cellobiose** or other reducing disaccharide be present. With the other common reducing sugars, the yellow persists or deepens to orange, as in Moore's test. Sucrose does not react.

Special Tests for Saccharides. The Copper-reduction Tests

(1) **Trommer's Test.**—To 5 ml. of solution add 2 drops of 5 per cent. copper sulphate and 10 drops of 20 per cent. sodium hydroxide. A light blue precipitate of copper hydroxide forms and may dissolve to form a blue solution if sufficient saccharide be present. Boil carefully. The blue changes to orange-red (cuprous oxide) if a reducing sugar be present. In the absence of reduction, the mixture turns black on prolonged boiling; for this reason excess of copper must be avoided, as the black coloration (cupric oxide) may obscure a slight red reaction.

✓ (2) **Fehling's Test.**—Mix equal parts of Fehling's reagent, A and B, about 2 ml. of each. A deep blue solution is formed. Boil for about a minute. No change is observed. Add an equal volume of the solution to be tested, and boil again. An orange-red precipitate forms if a reducing sugar be present.

Benedict's Qualitative Test.—Add 8 drops of the solution to 5 ml. of Benedict's qualitative reagent. Boil for one and a half minutes, or, preferably, place in boiling water for two to three minutes. Remove, and allow to cool. A green turbidity with a yellow precipitate indicates 0.1–0.3 per cent. reducing sugar in the original solution. A dense orange precipitate with a clear supernatant fluid indicates more than 1.5 per cent. of sugar.

(a) The reagent is designed specifically for the detection of sugar in urine. It consists of : 17.3 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, with 173 gm. Na citrate, and 100 gm. anhydrous Na_2CO_3 in 1 litre water.

(b) Benedict's qualitative reagent must not be confused with his quantitative reagent, which is used only for sugar estimation.

(3) **The Methylene Blue Test.**—Make 5 ml. of the solution alkaline with a few drops of 20 per cent. sodium hydroxide. Add a few drops of 0.1 per cent. methylene blue. Boil. The blue colour of the solution is discharged rapidly if a reducing sugar be present, but returns temporarily on aerating the contents of the tube by shaking.

(a) The test is not specific, and is almost too delicate to be of general application. The solution rapidly regains its colour on aeration owing to reoxidation of the dye precursor, leuco-methylene blue.

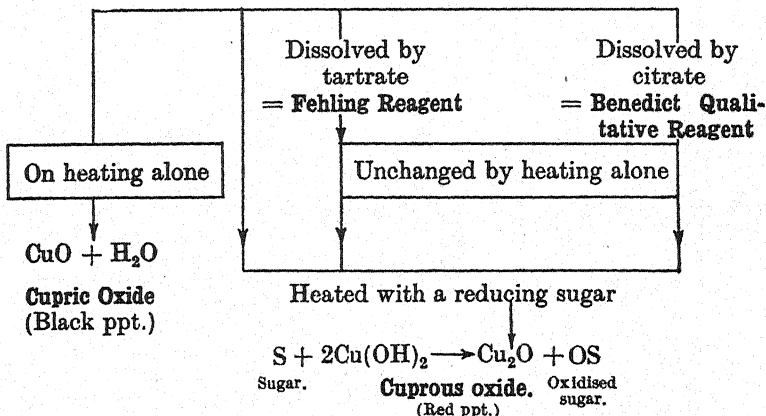
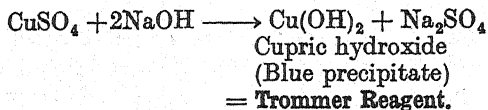
(b) The reaction is important in providing a useful "internal indicator" for copper reduction tests, such as that of Fehling, and simplifies the determination of an end-point in an estimation.

The above special tests are given by all monosaccharides and most of the compound saccharides, which in consequence are grouped as the **reducing sugars**. The commonest reducing sugars are lactose, maltose, glucose, fructose, galactose, mannose, and the pentoses ; sucrose is the only common non-reducing sugar.

Hydrolysis Test for Sucrose.—Apply Trommer's test to 5 ml. of sucrose solution. There is no reduction. Acidify the warm mixture with a few drops of concentrated hydrochloric acid till the colour changes to light green. Boil for half a minute. The acid hydrolyses the sucrose to glucose and fructose, both of which are reducing sugars. Add sufficient alkali (20 per cent. NaOH) to regenerate the Trommer mixture, as shown by the return of the original blue colour. Reduction now occurs if the solution be warm.

Other non-reducing carbohydrates, including starch, will give the test if boiled with the acid for several minutes.

MECHANISM OF THE COPPER REDUCTION TESTS



Notes on the Reagents.—(a) Trommer's reagent is unstable and must be prepared during the process of carrying out the test, which is delicate and reliable if properly applied.

(b) Fehling's reagent A is 34.65 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ in 500 ml. water. Reagent B is 60 gm. NaOH and 173 gm. NaK tartrate (Rochelle Salt) in 500 ml. water.

(c) The reagents are kept separate owing to the tendency of the mixture to become unstable and undergo spontaneous reduction when boiled, even in the absence of a reducing sugar. Reagent B after long exposure to light may give a positive result if used in the hydrolysis test for sucrose, irrespective of the presence of sugar.

(d) The tartrate is added to keep the copper hydroxide in solution and prevent the formation of a black precipitate of cupric oxide during boiling.

(e) When testing for traces of sugar it is advisable to dilute the mixed Fehling reagent 1 : 5, otherwise the deep blue may conceal a faint precipitate of cuprous oxide.

(f) When applied to urine the sensitivity of the test is lessened by the ammonia and creatinine, both of which dissolve cuprous oxide.

(g) If KSCN and $\text{K}_4\text{Fe(CN)}_6$ be present, as in *Benedict's quantitative reagent*, the red cuprous oxide does not form, being replaced by a white precipitate of cuprous thiocyanate. Hence, the end-point of the reaction is white.

(4) **The Ketose Test.**—To 3 ml. of sugar solution add about 2 ml. of concentrated hydrochloric acid. Boil carefully. An orange colour is given by sucrose and by its constituent saccharide, fructose. Since both these sugars contain a ketone group, :C-CO-C: , this

is a ketose test. Fructose is the only common *reducing* sugar that gives the test.

(5) **The Furfural Test.**—Mix 3 ml. of sugar solution with an equal volume of concentrated hydrochloric acid and 5 drops of 5 per cent. resorcinol in alcohol. Warm gently in a water-bath. A red colour is given at about 65° C. by pentoses, ketoses, and compound saccharides that liberate these sugars on hydrolysis. Aldoses only react in strong solution, and on prolonged boiling.

(a) This reaction, known as Seliwanoff's test, will distinguish fructose from all the other reducing sugars, and will distinguish sucrose from the other disaccharides.

(b) The reaction depends on the fact that aldoses do not liberate furfurals when boiled with the diluted hydrochloric acid, so if the test is being applied to a solid sugar it is always necessary to dilute the acid with an equal volume of water, otherwise the test is only a variant of the original general test for carbohydrates.

(c) Oxidation is necessary for the development of the colour, and the best results are obtained by using hydrochloric acid that has turned yellow owing to formation of chlorine, following exposure to light, or has been activated by addition of 1 per cent. FeCl_3 or H_2O_2 .

(d) By substituting other reactants for the resorcinol, it is possible to distinguish between pentoses, ketoses, and aldoses. Thus, using orcinol (Bial's test), pentose gives violet-blue; methylpentose and ketose turn orange-red; aldose does not react. Naphtho-resorcinol under similar conditions is used to detect glycuronic acid in urine and to distinguish it from true sugars, as the pigment obtained from a uronic acid differs from the pigment obtained from a sugar in being ether-soluble.

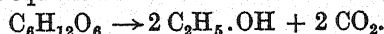
(e) Boiled for 2–4 minutes with an equal volume (3 ml.) of glacial acetic acid and a few drops of aniline ($\text{C}_6\text{H}_5\text{.NH}_2$), pentoses develop a red colour. None of the higher sugars give this test, as they require strong acids to liberate the furfurals.

SUMMARY OF THE FURFURAL REACTIONS FOR SUGARS

HCl.	Phenol, etc.	Pentose.	Ketose.	Aldohexose.	Glycuronic Acid.
15%	None	—	orange	—	—
35%	Thymol	purple	carmine	carmine	carmine
35%	α -naphthol	violet	purple	purple	purple
35%	Resorcinol	carmine	orange	orange	carmine
35%	Orcinol	blue	carmine	carmine	carmine
15%	Resorcinol	carmine	orange	—	carmine
15%	Orcinol	violet	orange	—	carmine
15%	Naphtho-resorcinol	purple	purple	—	purple
Acetic acid	Aniline	red	—	—	—

It is important to note that a fully concentrated aqueous solution of hydrochloric acid contains about 30–35 per cent. HCl. Hence, in carrying out tests requiring this concentration, the reagents and the sugar should be added in solid form or dissolved in a minimum amount of solvent, and excess of concentrated acid added. Acid of 15 per cent. concentration is obtained, approximately, by adding an equal volume of concentrated acid to the sugar solution. Excess of the phenol should be avoided, and heating is best done in a boiling water-bath.

✓ **Fermentation by Yeast.**—The yeast organism decomposes fermentable sugars into alcohol and carbon dioxide in accordance with the general equation :



(a) *Acid Formation.*—Shake up about 25–30 ml. of sugar solution with 2–3 gm. of baker's yeast (*Saccharomyces cerevisia*). Transfer about 5 ml. into a test tube, and add a few drops of the indicator phenol red. The reaction of the mixture is acid (yellow). Add a drop or two of N/10 NaOH until the mixture is just alkaline (red), and incubate the tube at 45°–50° C. for a few minutes. The contents gradually become acid owing to liberation of carbon dioxide from the fermenting sugar. On neutralising again, the process is repeated, and continues until all the sugar is fermented.

(b) *Alcohol Production.*—Fill the closed limb of a fermentation tube with the yeast-sugar mixture so as to leave no air bubbles. Place tube in an incubator at 40–50° for 1 hour, or leave for 24 hours at room temperature. If a fermentable sugar be present it is broken down into alcohol and carbon dioxide, which collects as a gas in the closed limb of the tube.

1. *Detection of carbon dioxide.*—Add 2–3 ml. of 20 per cent. sodium hydroxide to the tube; close the orifice, and invert the tube so as to mix the contents. The gas is rapidly absorbed by the alkali, and a negative pressure develops in the tube.

2. *Detection of alcohol.*—Unseal the tube. Add about 10 drops of an aqueous iodine solution, and mix by shaking. The iodine is decolourised by the alkali, and also interacts with any alcohol present to produce iodoform, CHI₃, which is recognisable by its smell.

Fermentable Sugars.—Four hexoses only are fermented by ordinary yeast, namely: *glucose*, *mannose*, *galactose*, and *fructose*. Of the commoner disaccharides, *maltose* and *sucrose* are attacked while *lactose* is not. Pentoses as a class are non-fermentable by yeast.

The test thus serves to distinguish pentoses and lactose from all other common reducing sugars. It is frequently applied to urine to distinguish *glucose* from (1) *lactose*, (2) *urinary pentose*, and (3) *glycuronic acid*, all of which reduce alkaline copper solutions.

Oxidation Tests.—Galactose and its derivatives (lactose, and galactosides) on oxidation by hot nitric acid yield mucic acid which is almost insoluble in cold water. Other saccharides yield saccharic

acid which is much more soluble, but forms an identifiable acid potassium salt. The following test, which must be carried out in a fume chamber, will detect both galactose and glucose, or sugars giving rise to them. It is not applicable to dilute solutions, which must be concentrated to syrups before oxidation.

(a) *Mucic acid*.—Add 10 ml. of strong nitric acid to about 2 gm. of lactose (or the sugar under examination) and 5 ml. of water in an evaporating dish. Heat carefully on a water-bath until a vigorous reaction begins, marked by the evolution of red fumes of nitrogen peroxide. Remove the dish, and when the reaction has subsided, resume heating until most of the acid has been expelled and a syrup remains. Rinse the syrupy residue into a test tube by means of not more than 10 ml. of hot water. Allow to cool. A white crystalline precipitate of mucic acid separates out. Microscopically it appears as clusters of short, bright prisms, which dissolve readily in ammonium hydroxide.

(b) *Saccharic Acid*.—Remove the precipitated mucic acid (if any forms) by filtration, add about 3–5 gm. of anhydrous potassium carbonate to the filtrate, and heat to ensure saturation. Pour the contents of the tube into a beaker, carefully acidify with 3–5 ml. of glacial acetic acid. On stirring and cooling the mixture, a white precipitate of potassium hydrogen saccharate separates out, and may be identified by its microscopic appearance of rosettes of sharp, brilliant needles.

If the first stage of the test yields no mucic acid it indicates that neither galactose nor lactose was present in the original material.

The Phenylhydrazine Test for Sugars.—Fill up about 1 cm. of a clean test tube with solid phenylhydrazine hydrochloride. Add twice as much solid sodium acetate. Then add 10 ml. of the sugar solution, and shake well. Heat the tube over a flame until the reagents are completely dissolved. Immerse the tube in boiling water, leave undisturbed for forty to sixty minutes, remove the flame, and let the tube cool slowly. If the reaction be positive the contents of the tube will have turned bright yellow, and a yellow crystalline sugar derivative, or osazone, will separate out as the tube cools.

Transfer some of the crystals by pipette to a slide, put on a cover slip, and examine microscopically.

Characteristics of the Commoner Osazones.—*Glucose, fructose, and mannose* yield the same osazone, **glucosazone**, which crystallises in yellow brushes or sheaves of slender needles; m.p. 205° C.

Galactosazone forms elongated strips and plates; m.p. 214° C.

Maltosazone occurs in stellate clusters of broad-bladed crystals; m.p. 205°–206° C. **Lactosazone** forms close tufts of short, fine crystals; m.p. 200° C. It is fairly soluble in hot water, and only separates out slowly on cooling. Osazone preparations from

disaccharides are liable to be contaminated by monosaccharides liberated by hydrolysis during prolonged boiling. Among the pentoses, **arabinosazone** occurs in long curved threads and wisps; m.p. 167° C., while **xylosazone** occurs in long needles; m.p. 115°–158° C. Non-reducing sugars, such as sucrose and raffinose, do not yield osazones, owing to the absence of an aldehyde group. The osazones can be identified by microscopic inspection, but it sometimes is necessary to find the melting point.

(a) Failure is often due to lack of sufficient phenylhydrazine; three molecules of which are required for each molecule of sugar (one for hydrazone formation, one for oxidation of an alcohol group in the hydrazone, and one for subsequent osazone formation).

(b) Owing to the instability of the free base, the hydrazine is used in the form of a hydrochloride. Since this is too acid, the mixture must be partly neutralised by addition of excess of sodium acetate.

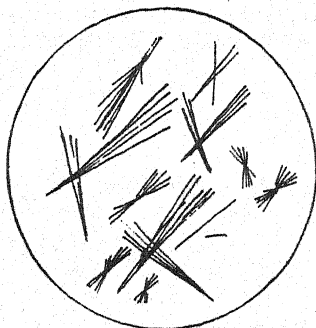
(c) If no precipitate appears in the tube after 60 minutes' heating, repeat the process for another 30 minutes, and let the tube cool slowly over-night. Glucose and fructose yield osazones much more rapidly than either maltose or lactose. If the mixture does not turn yellow after being heated, no osazone-forming carbohydrate is present.

(d) The advantage of the osazone test lies in its ability to reveal the presence of different sugars in a mixture, as well as to identify individual sugars.

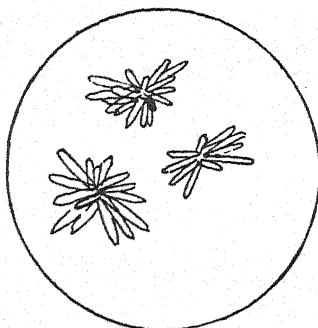
Summary of the Reactions of the Commoner Carbohydrates

Carbohydrate.	TEST.								
	Thymol.	Iodine.	Nitro-chromic.	Cu Reduction.	Osazone.	Ketose.	Yeast Fermentation.	Polarimetric Rotation.	Methylamine.
	1	2	3	4	5	6	7	8	9
Pentose .	+	—	+	+	+	±	—	d or l	—
Hexose :									
glucose .	+	—	+	+	G	—	+	d	—
fructose .	+	—	+	+	G	+	+	l	—
galactose .	+	—	+	+	Gl	—	+	d	—
Dihexoside :									
maltose .	+	—	+	+	M	—	+	d	+
sucrose .	+	—	+	—	—	+	+	d	—
lactose .	+	—	+	+	L	—	—	d	+
Hexosan :									
cellulose .	+	±	—	—	—	—	—	(d)	—
starch .	+	blue	—	—	—	—	—	d	—
e-dextrin .	+	red	—	—	—	—	—	d	—
glycogen .	+	red	—	—	—	—	—	d	—
inulin .	+	—	—	—	—	+	—	l	—

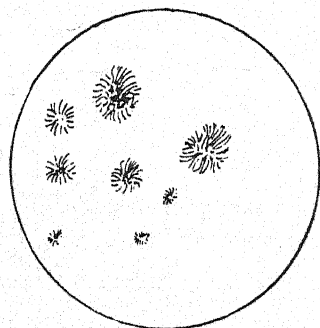
G = glucosazone; Gl = galactosazone; M = maltosazone; L = lactosazone.
± = reaction varies with conditions



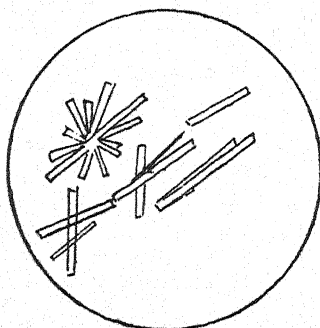
GLUCOSAZONE (X 50)



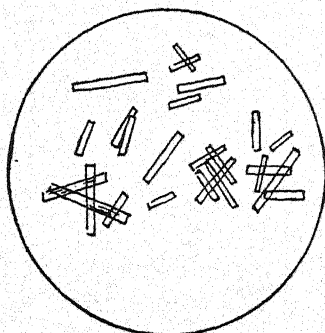
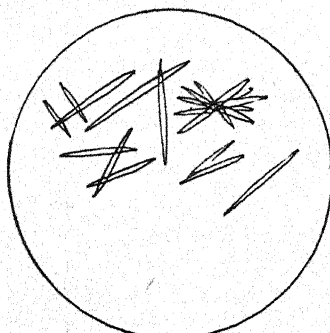
MALTOSAZONE (X 200)



LACTOSAZONE (X 200)



GALACTOSAZONE (X 200)

MUCIC ACID
(X 50)POTASSIUM HYDROGEN
SACCHARATE
(X 50)

METHODS OF SUGAR ESTIMATION.

A. Copper Reduction.

For sugars in dilute (0.1–4.0 per cent.) solutions.

B. Yeast Fermentation.

For all common sugars, except lactose and pentoses.

A. Copper-reduction Methods.

I. Fehling's Method. (Blue → Red)

(1) Measure carefully by pipette 5 ml. of Fehling's reagent A into a 150 ml. conical flask. Add 5 ml. of reagent B, using a measuring cylinder to avoid contaminating the pipette. Add about 40–50 ml. of water. Set to boil over a small flame.

When several estimations are being done, it is convenient to mix a quantity of the reagents A and B in equal volumes beforehand. 10 ml. of the mixture are measured by pipette into the flask and diluted with 50 ml. of water.

(2) Fill a clean burette with the sugar solution. Run out sufficient to free the delivery tube of air bubbles. Note reading of burette.

(3) When the Fehling's reagent is boiling, run in the sugar solution, 0.5–1.0 ml. at a time, at short intervals, until the blue colour of the mixture changes to orange-brown owing to formation of cuprous hydroxide.

(4) Detection of the End-Point. The end-point is reached when all the blue colour has been discharged. This may be hard to detect in composite liquids, such as urine. Remove the flame, and let the mixture cool. As the precipitate subsides, observe the clear solution.

Blue = underdone.

Colourless = done.

Yellow = overdone.

Resume titration.

Note reading of burette, and find volume of sugar added.

Note reading of burette. Start a fresh titration, and add less sugar solution.

Lane and Eynon Method for Determining the End-Point.—At the last stage of the reaction add a few drops of 1 per cent. methylene blue. This colours the mixture a dark blue, which persists until all the copper has been reduced. Then, the methylene blue in turn is reduced, and the colour of the solution clears rapidly. This change represents the end-point, the pigment being used as an internal indicator. It is important not to shake the flask during the latter stages of the reduction, as the leuco-methylene blue is easily re-oxidised by contact with air.

(5) *Calculation.*—10 ml. of mixed Fehling's reagent are completely reduced by 0.05 gm. of glucose. Hence, the volume of sugar solution used in the titration contains 0.05 gm. of glucose, or its equivalent.

where x = percentage of glucose in the solution.
 N = number of ml. of solution required for complete reduction.

$$\text{Or, } x = \frac{5}{N}$$

Notes.—(a) The estimation should be done at least twice, for accuracy.

(b) A small piece of "chalk" may be added if the boiling mixture begins to spurt.

(c) Mixed Fehling's reagent deteriorates on long keeping, and is stored separately as two solutions.

(6) *Calculation for Reducing Sugars other than Glucose.*—Should the reducing sugar be other than glucose an alteration must be made in the calculation formula, on account of the fact that the copper equivalent differs for different sugars.

(7) *Estimation of a Non-reducing Sugar such as Sucrose.*—The sucrose must first be hydrolysed into its constituents, glucose and fructose, the equimolecular mixture being termed "invert sugar." This may be done by enzymes or dilute acids under specified conditions, as described by Hinton ("Recent Advances in Analytical Chemistry." Ed. Mitchell). The result is expressed either as percentage of invert sugar, x (invert sugar) $= \frac{5.1}{N}$, or as sucrose, knowing

that 1.00 gm. invert sugar = 0.95 gm. sucrose.

To ensure that all the sucrose present has been "inverted," it is usual to repeat the hydrolysis for a longer interval of time, and see if the final results are the same.

Factor :	10 ml. of Fehling's reagent are reduced completely by :
x (glucose) $= \frac{5}{N}$	0.05 gm. glucose.
x (fructose) $= \frac{5.3}{N}$	0.053 gm. fructose.
x (lactose) $= \frac{6.76}{N}$	0.0676 gm. lactose.
x (maltose) $= \frac{7.4}{N}$	0.074 gm. maltose.

II. Benedict's Method. (Blue \rightarrow White)

(1) Measure carefully by pipette 25 ml. of Benedict's quantitative reagent into a 150 ml. flask. Add 3–5 gm. of anhydrous sodium carbonate (or as much as will fill 2 cm. of an ordinary test tube), and a small piece of "chalk" to keep the mixture from spurting.

(2) Boil the mixture over a moderate flame, and while boiling add the sugar solution from a burette, as in Fehling's method.

(3) The end-point is shown, as in the micro-method, by the replacement of all the blue colour by pure white. If it turns yellowish, too much sugar has been added.

Benedict's method has a sharper end-point and a greater specificity than Fehling's method. Its chief disadvantage is the liability of the mixture to spurt and boil over, unless the estimation be carried out rapidly, carefully and attentively.

(4) *Calculation.*—Benedict's reagent is of such a strength that 25 ml. are reduced by 0.05 gm. glucose (or, 25 ml. Benedict's reagent = 10 ml. Fehling's reagent). As before, $x = \frac{5}{N}$.

Where N = number of ml. of sugar solution added ;

x = percentage of glucose.

For the other reducing sugars the appropriate equation must be used.

(a) It is necessary to keep Benedict's solution boiling during the estimation, adding water if the mixture becomes very concentrated, and starts to spurt.

(b) The use of methylene blue to sharpen the end-point is inadvisable. The Benedict reaction is marked by a colour change and not by the subsidence of a precipitate.

(c) In titrating sugar solutions weaker than 0.3 per cent., use 10 ml. reagent and 10 gm. anhydrous carbonate.

Here, x (glucose) = $\frac{5}{2.5 N} = \frac{2}{N}$.

III. The Drop Method

To avoid the difficulties, due to super-heating and spurting, which unpractised workers encounter when using Benedict's method, the following modification has been made. It is rapid, and can be carried out by means of a calibrated pipette instead of a burette.

(1) Fill up about 1 cm. of a clean test tube with anhydrous sodium carbonate. Add exactly, by pipette, 5 ml. of Benedict's *quantitative* reagent.

(2) Boil gently until most of the carbonate is dissolved.

(3) Add the sugar solution from a burette or a pipette fitted with a rubber bulb, one drop at a time. Boil for a few seconds after addition of each drop. Count the drops.

(4) When the blue colour has almost gone, proceed slowly, and let the tube cool in the rack for about one minute before addition of the next drop.

(5) The end-point is obtained when the mixture is pure white. If excess of sugar has been added, the mixture becomes yellow. The average time of an estimation should not exceed three to four minutes. The estimation should be repeated.

(6) *Calculation.*—5 ml. of Benedict's quantitative reagent are reduced by 0.01 gm. of glucose. Hence, the solution added contains 0.01 gm. of glucose, or its equivalent.

$$x \text{ (glucose)} = \frac{d}{n}; \quad \begin{array}{l} \text{where } x = \text{percentage of glucose in the solution.} \\ n = \text{number of drops required for complete} \\ \text{reduction.} \end{array}$$

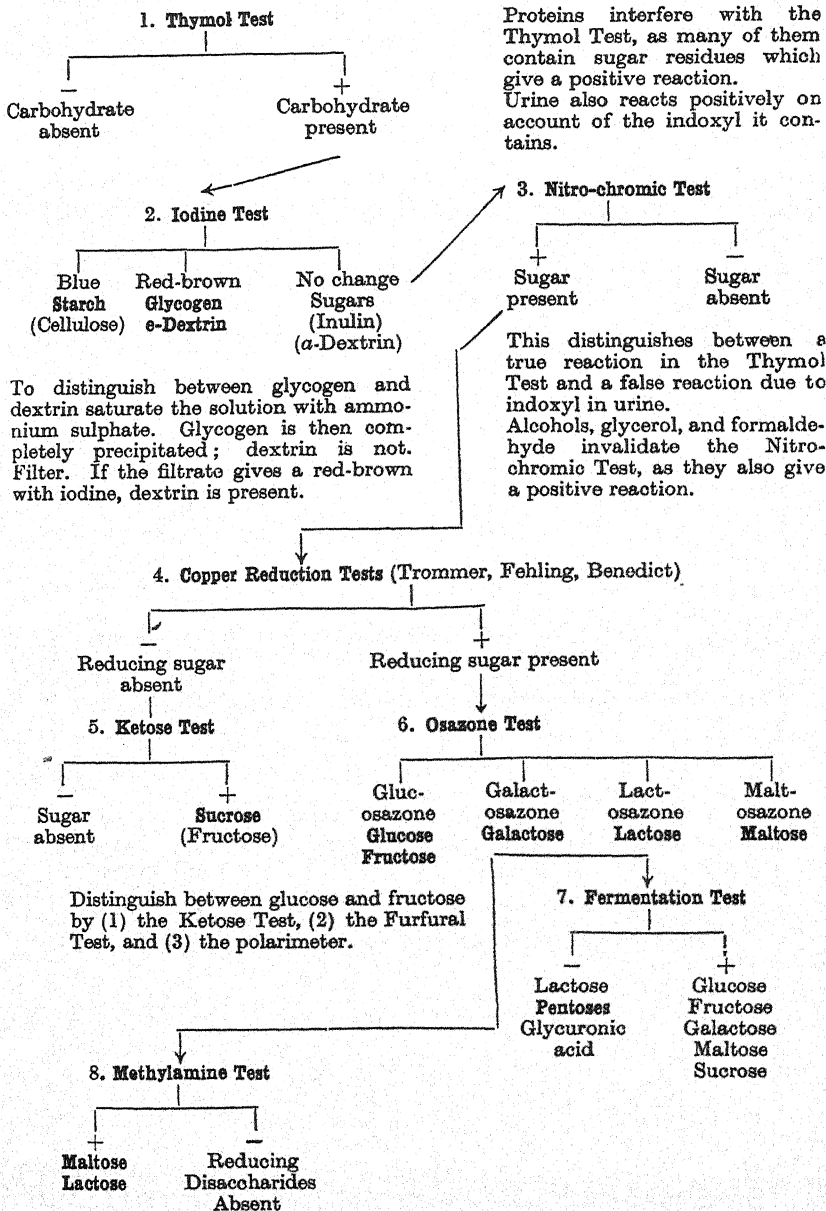
$$d = \text{number of drops in 1 ml., usually 20.}$$

$$x \text{ (lactose)} = \frac{1.35d}{n}; \quad x \text{ (maltose)} = \frac{1.48d}{n}.$$

Note.—The value for d can be found experimentally by counting the number of drops delivered from a known volume of the solution in the pipette or burette. It ranges from 18–21 per ml., and 20 is an average value for a burette.

Estimation of Sugar in Concentrated Solution.—If the solution contain more than 5 per cent. of reducing sugar (i.e., if less than 1 ml. reduces all the copper reagent), it must be diluted 1 : 5 or 1 : 10 to yield an accurate result with either Fehling's or Benedict's method. For dilution 1 : 10, measure 10 ml. of sugar solution exactly by pipette into a 100 ml. measuring flask. Fill up carefully to the 100 mark. Mix thoroughly. Rinse out the burette twice with the diluted sugar solution. Fill the burette, and estimate as before. The final result must be multiplied by 10 to obtain the percentage of sugar in the original undiluted solution.

Method for Identifying One of the Common Carbohydrates



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CHAPTER 8

PROTEINS

Definition.—Proteins are complex natural compounds formed by the condensation of mixed amino acids. They are present in all plant and animal tissues, and represent the stable organic form assumed by nitrogen during its association with the living organism.

Class Characteristics.—(1) All proteins contain C, H, O, and N, usually in the following amounts, expressed as percentage :—

C, 50-55 ; O, 21-23.5 ; N, 15-17.6 ; H, 6.5-7.0.

The high content of nitrogen is a feature of the protein family of biological compounds.

Many proteins also contain S and P, depending on the particular amino acid units or esters present.

(2) All proteins are of high molecular weight and colloidal dimensions.

(3) All proteins give a rose-violet colour on addition of dilute copper sulphate and sodium hydroxide. This is the copper protein or "biuret" test.

(4) All proteins on hydrolysis yield mixtures of α -amino acids, the type formula of which is $R \cdot CH(NH_2) \cdot COOH$.

(5) With the exception of the group of pigmented chromoproteins, the proteins as a family have no characteristic colour, odour, or taste.

Proteins are classified *biochemically*, according to solubility, coagulability, and amino acid content ; and *biologically*, according to natural distribution and function.

BIOCHEMICAL CLASSIFICATION OF PROTEINS

A	B	C
Simple Proteins	Conjugated Proteins	Derived Proteins
(1) Protamines.	(8) Phosphoproteins	(15) Denatured Proteins
(2) Histones	(9) Nucleoproteins	(16) Cleavage Products :
(3) Gliadins	(10) Glycoproteins	(a) Metaproteins
(4) Glutelins	(11) Chromoproteins	(b) Proteoses
(5) Sclerins	(12) Zymoproteins	(c) Peptones
(6) Albumins	(13) Hormoproteins	(d) Peptides
(7) Globulins	(14) Autogenic proteins	(e) Amino acids, etc.

CLASS PROPERTIES OF THE PROTEINS

A. SIMPLE PROTEINS

(1) **Protamines.**—The simplest natural proteins, found only in fish sperm. At least twelve protamines are known, each being made up of four to ten different species of amino acid, one of which is always arginine. Thus *salmin* (from salmon sperm) has a minimal molecular weight of 2,855, and on hydrolysis liberates 14 molecules of arginine, 3 of proline, 3 of serine, and 1 of valine. All protamines are water-soluble and strongly basic, owing to their high content of diamino acids.

(2) **Histones.**—Basic proteins resembling protamines, but containing a greater variety of amino acid units. They occur usually in union with nucleic acid.

Examples are :—

- (a) *Thymus histone*, a powerful anti-coagulant of blood, originally isolated from the thymus gland of the calf.
- (b) *Nucleohistones* from cell nuclei of various tissues, including sperm cells.
- (c) *Globin*, the protein component of the blood pigment, hæmoglobin.

(3) **Gliadins or Prolamins.**—These are peculiar to plants, and occur largely in the seeds of cereals. They are insoluble in water and in 90 per cent. alcohol, but have the special property of being soluble in 70–80 per cent. aqueous alcohol. They are not coagulated by heat. Important prolamins are : hordein, from barley ; zein, from maize ; and gliadin, from wheat and rye.

As a class, gliadins are characterised by their high content of proline, which accounts for the original name prolamine.

(4) **Glutelins.**—Vegetable proteins found chiefly in cereal grain. They are insoluble in water and aqueous alcohol in all concentrations, but may be dissolved by dilute acids or alkalis. They are not coagulated by heat. *Glutenin*, from wheat, and *oryzenin*, from barley, are the most definite members of the class.

When wheat flour is made into a paste with water the gliadin present forms an adhesive and binds together the glutenin. The mixture is termed *gluten*, and is responsible for the production of dough. Gluten makes up about 6–12 per cent. of wheat flour.

(5) **Sclerins, Scleroproteins, or Albuminoids.**—Insoluble proteins found in the skeletal, connective, and epidermal tissues of animals. They do not occur in plants. Scleroproteins are very stable, and resist acid and alkaline hydrolysis, but ultimately are resolved into amino acids.

- (i.) *Collagens* or *gelatin precursors*, found in bone, cartilage, and the "white fibres" of connective tissue. Collagens are hydrolysed by boiling with concentrated acids or alkalis, or by superheated steam. They are attacked by the gastric enzyme pepsin, but not by trypsin. *Gelatin* is the characteristic product of intermediate hydrolysis. Collagens are rich in the simple amino acid glycine, the yield of which may be as high as 25 per cent. They lack the biologically important amino acids, cystine, tyrosine, and tryptophane.
- (ii.) *Elastins*, found in the "yellow" or elastic fibres of connective tissue, in cartilage and ligaments. They differ from collagens in being readily attacked by both pepsin and trypsin. The end-products of hydrolysis are especially rich in glycine (25 per cent.) and leucine (20 per cent.).
- (iii.) *Keratins*.—These scleroproteins are characteristic of epidermal tissue, and occur in hair, wool, feathers, claws, horns, and nails. They are the most stable of all proteins, and are well adapted to withstand the environment. Insoluble in water, dilute acids and alkalis, they resist proteoclastic enzymes, including pepsin and trypsin.

Keratins are dissolved by concentrated alkalis, and by metallic sulphides, a property that underlies the use of barium or calcium sulphide as a depilatory. Chemically, true keratins are rich in the amino acids histidine, lysine, and arginine, which are present in the ratio 1:4:12. The tyrosine and cystine contents also are usually high; tyrosine being about 3-4 per cent., and cystine reaching values of 8-14 per cent. in wool, and 15-20 in human hair.

(6) *Albumins*.—The group of water-soluble, heat-coagulable proteins found in animals and plants. Important members are *ovalbumin*, from egg-white; *serum albumin*, from blood; *lactalbumin*, from milk; *myoalbumin*, from muscle, *leucosin*, from wheat, rye, and barley; *legumelin*, from pea, bean, and lentil.

As a class they undergo a characteristic irreversible coagulation when heated to about 75° C. in solution. Coagulation is most marked in slightly acid solutions (pH6-pH5), and is retarded by alkalis. It forms an important test for higher proteins.

- (i.) *Ovalbumin* makes up the greater part (10-13 per cent.) of egg-white. It can be obtained in crystalline form by half-saturation with ammonium sulphate at pH 4.58. It is lævo-rotatory in solution, $[\alpha] = -35.5^\circ$.
- (ii.) *Serum albumin* occurs in serum (4-6 per cent.), lymph, and other tissue fluids. Like ovalbumin it coagulates about 75° C., but differs in being more lævo-rotatory, $[\alpha] = -56^\circ$.

The vegetable albumins resemble serum albumin in most properties, but differ in that they contain glycine, an amino acid that is often absent from animal albumins, other than lactalbumin.

(7) **Globulins.**—Heat-coagulable proteins, insoluble in pure water but soluble in presence of electrolytes. They accompany albumins in many tissues, and carry corresponding names: *ovoglobulin*, *serum globulin*, *lactoglobulin* and *myoglobulin*. *Fibrinogen*, from blood plasma, is a globulin, and the majority of vegetable proteins and seed proteins are of the globulin type. Examples are: *legumin*, from seeds of pea, bean, and lentil; *excelsin*, from Brazil nut; *edestin*, from hemp seed. Reserve protein of seeds is usually a globulin, many can be crystallised.

Distinction between Globulins and Albumins.—Globulins, unlike albumins, are insoluble in pure water, and must be extracted from tissues by means of dilute (5 per cent.) salines, such as NaCl, MgSO_4 , etc. Removal of the salt by dialysis precipitates the globulin. All animal globulins precipitate on full saturation with MgSO_4 , or on half-saturation with $(\text{NH}_4)_2\text{SO}_4$, vegetable globulins, however, are not completely precipitated by these reagents. Albumin and globulin usually occur together in animal tissues and fluids, such as blood serum. Separation may be effected by:—

- (1) Addition of excess of water, which precipitates the less soluble globulin.
- (2) Dialysis, which precipitates the globulin by removing the salts that keep it in solution.
- (3) Addition of an equal volume of saturated (*i.e.*, 80 per cent.) ammonium sulphate; this half-saturates the protein solution, and precipitates the globulin.

None of these methods can be considered as exact, the sharpness of the separation depending on many factors, such as the pH of the mixture and the relative concentrations of proteins present. "Salt-ing-out" is a process of dehydration, which removes the water-envelope of the hydrophil protein emulsion particles, leaving an unstable suspensoid.

The Albumin-Globulin Complex.—Albumins and globulins are distinguished from all the other proteins by being coagulated by heat; apart from this, their individual characteristics depend largely on the method employed to separate them from one another, and it is believed that they occur naturally in the form of an unstable association, or protein complex, making up the colloidal matrix of cells and tissues, including the blood plasma. The term *orosin* is sometimes used to denote this heat-coagulable protein complex.

B. CONJUGATED OR COMPOUND PROTEINS

These consist of proteins united to a non-protein, or *prosthetic*, group.

(8) **Phosphoproteins.**—Compounds of protein and phosphoric acid. The phosphoric radicle is united in two ways: (i) as a peptide linkage, —NH—OP— , attacked by trypsin; and (ii.) as an ester linkage, —CO—OP— , attacked by phosphatase. Phosphoproteins are found in milk and in egg-yolk, and are of primary importance in the nutrition of the young animal. They are characterised by the presence of all the essential amino acids, and by their high content of glutamic acid, which may reach 20 per cent.

The chief phosphoproteins are:—

- (a) **Caseinogen**, a characteristic constituent of all milk, the value ranging from about 1 per cent. in human milk up to nearly 9 per cent. in the milk of the cat. Caseinogen is insoluble in water, but readily dissolves in dilute alkalies to form soluble caseinates. Caseinates may be precipitated unchanged by addition of excess of alcohol, or they may be decomposed by addition of dilute acid, whereupon the liberated caseinogen is precipitated.

This occurs when milk becomes acid, or “sour,” owing to bacterial decomposition of lactose to lactic acid.

Caseinogen is precipitated completely about pH4.6, if more acid be added the protein redissolves.

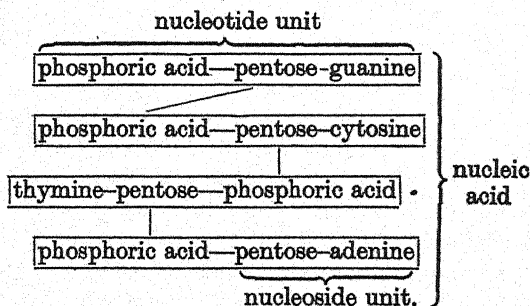
Caseinogen solutions are not heat-coagulable, but on the addition of the enzyme *rennin*, the protein is changed into another form, “casein,” or paracasein, which yields an insoluble coagulum with calcium salts. This coagulation of milk occurs in the stomach as a preliminary stage in the digestion. It is distinguished from the clotting of sour milk by being (i.) irreversible, and (ii.) due to the action of a special enzyme, the chymase, or rennin, in gastric juice.

In British nomenclature, *caseinogen* denotes the soluble protein, and *casein* is the insoluble form; in American and other nomenclatures *casein* is the soluble, and *paracasein* the insoluble protein. Pending the long-overdue agreement on nomenclature, the soluble form of “casein” may be termed *caseinogen*, and the derived form produced by rennin may be termed *paracasein*, thus avoiding the use of the ambiguous *casein*.

- (b) **Vitellin**, the chief protein of avian egg yolk, is a phosphoglobulin, being heat-coagulable, and insoluble in water but soluble in dilute salines.
- (c) **Livetin**, another phosphoglobulin, makes up 20–25 per cent. of the total yolk protein (or 0.6–0.9 gm. livetin) in the egg of the domestic fowl.

(9) **Nucleoproteins.**—The nuclei of cells are characterised by the presence of strands of a material staining deeply with basic dyes, and for this reason termed *chromatin*. Chromatin is the carrier of the genes, or inheritance factors, and is composed partly of nucleoprotein, a compound of histone or protamine and nucleic acid. The protein component differs in the different species of plants and animals, and is a determinant of biological individuality.

Nucleoproteins are almost insoluble in water, but dissolve in alkalis, and by this means can be extracted from tissues rich in nuclei, such as thymus and pancreas. They were obtained originally from pus, which is an emulsion of white corpuscles. Another rich source is the sperm of fishes. On acid or enzyme hydrolysis, nucleoproteins are resolved into protein and nucleic acid, which further hydrolysis converts into four units, or **nucleotides**. Each nucleotide is the phosphoric ester of a **nucleoside**, or pentose derivative of an amino purine (*adenine* or *guanine*), or an amino pyrimidine (*cytosine* or *methyl cytosine*).



Thus, a nucleic acid is a mixed tetranucleotide, and is derived from: two purines, two pyrimidines, four molecules of a pentose and four molecules of phosphoric acid.

The pentose is either D-ribose, $C_5H_{10}O_5$, or D-2-desoxyribose, $C_5H_{10}O_4$, and accordingly two types of nucleic acid are known.

1. *Thymo-nucleic acid*, found in thymus, spleen, pancreas, liver, kidney, fish sperm, and other sources of animal chromatin. In this form of the acid the pyrimidines are cytosine and thymine and the sugar is desoxyribose, which gives a positive Feulgen colour test (p. 407).

2. *Yeast nucleic acid*, found in yeast, wheat germ and other sources of plant chromatin, yields the pyrimidines cytosine and uracil, and the sugar D-ribose, which does not give Feulgen's test.

It was formerly thought that yeast nucleic acid was the typical plant nucleic acid and thymo-nucleic acid was only found in animals, but this distinction has had to be abandoned on account of the discovery of the ribose nucleic acid in many animal tissues.

Ribose nucleic acid has no free phosphoric side-chain, and is probably a closed-ring structure, or cyclic tetranucleotide. Desoxyribose nucleic acid is more complex, and is a polymer of high molecular weight.

According to the Stedmans (1943-44), the average percentage composition of dried nuclear material is : chromosomin, 56-60 ; nucleic acid, 28-34 ; histone, 12-16. The basic protein, histone, is combined with nucleic acid, with which it forms a nucleoprotein, and with **chromosomin**, an acidic protein, characteristic of the chromosomes, and staining violet with the nuclear stain hæmatoxylin.

(10) **Glycoproteins or Mucoproteins.**—Compounds of protein and neutral mucopolysaccharides. The glycoproteins were formerly classified as mucins and mucoids, but the term "mucin" is ambiguous and applies to many viscous secretions, some of which owe their properties to free mucopolysaccharides. Glycoproteins are represented by : ovomucoids from egg white, serum mucoid, thyroglobulin, and the gonadotrophic hormone found in the urine of pregnancy.

Glycoproteins dissolve in water to form very viscid solutions not coagulated by heat. They resist the attack of ordinary digestive enzymes, but are hydrolysed by hot alkalies, with liberation of hexosamine from the mucopolysaccharide residue (p. 110).

(11) **Chromoproteins.**—Compounds of protein and a pigment, usually a metallic derivative of porphyrin.

(i.) *Hæmochromes*, or blood pigments, the chief of which is **hæmoglobin**, the red chromoprotein found in the blood cells of all vertebrates. It is composed of 94 per cent. of a histone, *globin*, united to the ferrous porphyran, *hæm*. Chlorocruorin is a green chromoprotein found in some marine worms. Hæmocyanin occurs as a blue hæmochrome in crustaceans.

(ii.) *Cytochromes*, hæm complexes found in all aerobic cells, and forming part of the cell oxidation apparatus.

(iii.) *Phytochromes* : plant chromoproteins, including phycoerythrin, a red pigment, and phycocyan, a blue pigment, both found in seaweeds.

(iv.) *Flavoproteins.*—The "yellow" respiratory catalyst, discovered by Warburg, is a chromoprotein of D-riboflavin (vitamin B₂).

(12) **Zymoproteins.**—Heat-labile proteins having catalytic properties. This group includes the enzymes, with possible exception of taka-diastase and emulsin, which are not destroyed by protein-splitting catalysts.

(13) **Hormoproteins.**—Several of the internal secretions of the human body have been found to be proteins or protein derivatives. Chief among these protein hormones are : insulin, secretin, thyroglobulin, prolactin and other pituitary factors, parathyrin.

Autogenic Proteins.—Some proteins appear to have the power of self-reproduction, or autogenesis, within the living cell; the protein molecule being able to act as a stimulus and a pattern for cell metabolism. An example of a self-reproducing protein is the chromatin of the cell nuclei, which, during the development of the organism, is able to reproduce and distribute itself in all the cell nuclei of the animal or plant.

Virus Proteins.—In 1876, Pasteur showed that diseases in animals can arise from infection by micro-organisms, which vary in shape and size, from 1μ (micrococci) up to 30μ or more (spirochaeta). Being of these dimensions, they are visible by high-power microscopy (600 to 1,000 magnifications), and are retained in suspension when filtered through unglazed porcelain. About 1880, while investigating *rabies*, Pasteur was led to infer that some diseases might be due to organisms too minute to be seen by the optical microscope. This was confirmed, in 1892, by Ivanoski, who showed that sap from plants with "leaf-mosaic" disease could infect other plants, even after it had passed through filters fine enough to retain all known bacterial types. The term *virus* was used to denote these filterable pathogenic agents, and it is now established that viruses are responsible for yellow-fever, small-pox, foot-and-mouth disease, and many other infections of animals and plants. In 1935, Stanley isolated in crystalline form the virus of tobacco mosaic disease, and, in 1936, Northrop isolated *bacteriophage*, the virus capable of destroying living bacteria.

Tobacco mosaic virus is a protein of high molecular weight (17×10^6), and contains most of the common amino acids.

Bacteriophage, as isolated from dissolved cultures of streptococci, resembles a nucleoprotein. Virus are separated from associated material by fractional precipitation at appropriate pH in a manner similar to that adopted for the isolation of certain enzymes. The diameter of these viruses, as calculated from ultra-filtration and sedimentation data, ranges from 10μ (foot-and-mouth disease) up to 175μ (vaccinia). By means of the electron microscope, the finer details of their forms are being elucidated.

Biological Status of the Viruses.—Whether the virus be regarded as the smallest known type of life, an organism that through parasitism can only function when within the living cell, or whether it be regarded as a non-living colloid with unique properties, depends on the concept of "life," a problem that is metaphysical rather than physical. To the biochemist, the simple virus seems to be a non-living molecular type, capable of evoking self-reproduction, and thus perpetuating itself, at the expense of living tissue. Separated in crystalline form or in sterile solution, it is inert, and shows

none of the properties of respiration, growth, or reproduction that constitute the biochemical criteria of life. That is to say, it does not require continuous transformation of energy for the maintenance of its identity, and biological potency, and, in this respect, differs from seeds, which, as long as they are capable of germination, show a minute but measurable release of carbon dioxide.

C. DERIVED PROTEINS

Products obtained by the denaturation and cleavage of natural proteins. They represent stages in protein digestion and synthesis.

(14) **Denatured Proteins.**—Denaturation is marked by complete loss of solubility in water and in neutral salt solutions. It is usually irreversible, and is brought about by many agents, including heat, strong acids, and the prolonged action of alcohol. Denaturation may be effected also by specific catalysts, as in coagulation of caseinogen by rennin, or the conversion of fibrinogen into insoluble fibrin during the clotting of blood.

Heat-Coagulation.—Albumins and globulins differ from all the other proteins in being coagulated when heated in aqueous solution. The change is preceded by heat-denaturation, which is favoured by an acid reaction; then the insoluble protein particles flocculate or coagulate, a change which takes place best in presence of neutral electrolytes, such as NaCl, about pH 5·7–pH 5·9.

Heat-coagulation will not take place in an alkaline solution, or in a strongly acid solution deficient in salts. Hence, in testing for albumin or globulin the solution is first slightly acidified with dilute acetic acid, and treated with sodium chloride. The coagulation temperature depends on the nature of the protein, the reaction of the mixture, and the electrolytes present.

Blood serum at pH 5·7 coagulates about 75° C.; egg-white coagulates about 62° C. Heat-coagulation is an important group test, and a means of detecting traces of higher protein in urine.

(15) **Protein Cleavage Products.**—In denaturation and coagulation the protein molecule is rearranged but not decomposed; in the subsequent changes of cleavage, the molecule is fragmented in stages until the final end-products are reached. Four intermediate and somewhat artificial levels of hydrolysis are recognised :—

- (a) *Metaproteins.*—Cleavage products not coagulable by heat, insoluble in water at pH 6·0, but soluble in greater concentrations of acid or alkali. When dissolved in either of these reagents they are termed acid metaprotein and alkali metaprotein, respectively. They resemble higher proteins in giving a violet colour reaction with copper sulphate and

sodium hydroxide. Metaproteins from albumin and globulin give a positive Thiol test (p. 166), owing to the unmasking of $-SH$ groups during early hydrolysis.

- (b) *Proteoses*.—Cleavage products not coagulable by heat, soluble in water, and not precipitated at pH 6.0, but completely precipitated by saturation with sodium sulphate at 33° C. Unlike metaproteins and higher proteins, the proteoses give a rose colour reaction in the copper protein test.
- (c) *Peptones*.—Cleavage products resembling proteoses, but not precipitated by saturation with sodium sulphate. They give a rose colour reaction in the copper protein test, and are completely precipitated by strong tannic acid. Peptones resist the action of the proteoclastic enzymes, pepsin and pure trypsin, but are attacked by the peptidases, such as erepsin, found in the small intestine.
- (d) *Peptides*.—Simple hydrolytic products, mostly soluble in water and not precipitated by tannic acid. They give a rose colour in the copper protein test, and are precipitated by excess of alcohol.

Proteoses, peptones and peptides constitute the sub-group of **lower proteins**, as distinct from their precursors, the higher proteins.

- (e) *Amino Acids*.—The chief end-products of protein hydrolysis. The type formula is $R.CH(NH_2).COOH$, and they are derived from simple aliphatic acids by replacement of H in the α -position by the *amino group*, NH_2 . Many amino acids carry other groups attached to the terminal C atom on the left end of the aliphatic chain. At least thirty different amino acids have been recovered from the products of protein hydrolysis.
- (f) *Imino Acids*.—Two imino acids, *proline* and *hydroxyproline*, have been isolated.
- (g) *Hexosamines*.—*Chondrosamine* and *chitosamine* occur among the products of glycoprotein hydrolysis.
- (h) *Ammonia* is an invariable product of alkaline hydrolysis, and is believed to come from the decomposition of structural units containing the amide group, $-CO.NH_2$.

Protein hydrolysis may be brought about by :—

- (i.) Boiling with strong acids, such as 5–25 per cent. HCl. This is the principal general method in use, although it involves loss of at least two important amino acids, namely, tryptophane and cystine.
- (ii.) Boiling with alkalis. This causes loss of at least three acids, citrulline, arginine, cystine, and, possibly, histidine.

- (iii.) Zymolysis or hydrolysis by enzymes. This is highly efficient within the organism, but slow and incomplete in the laboratory. It is mostly used in the preparation of the less stable amino acids, such as tryptophane.

The Carbohydrate Content of Proteins.—Egg albumin, serum albumin and many other proteins give a purple colour on being warmed with thymol and hydrochloric acid, showing the presence of a carbohydrate. This reaction persists even when the protein has previously been purified by repeated crystallisations, and has been traced to the constant presence of a sugar residue, mannose or galactose, in the protein molecule.

Hexose Content of Proteins

Egg-white protein :

ovoglobulin	.	4.0 per cent. mannose.
ovalbumin	.	1.7 per cent. mannose.
ovomucin	.	14.9 per cent. mannose + galactose.

Serum protein :

seralbumin	.	0.47 per cent. mannose + galactose.
serglobulin	.	1.82 per cent. mannose + galactose.

Milk proteins :

casein	.	0.31 per cent. galactose.
lactalbumin	.	0.44 per cent. galactose.

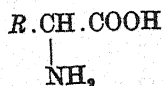
CHAPTER 9

AMINO ACIDS AND PROTEIN STRUCTURE

WHILE the number of natural proteins is not yet known, and must be at least as great as the total number of plant and animal species, since species individuality seems to reside in protein structure, the number of amino acids isolated from natural sources is about forty, and of these, twenty-five are generally accepted as protein structure units. Variety in protein architecture is obtained by variety in the content and order of arrangement of these units within the protein molecule.

Definition.—An amino acid is an organic acid in which one or more residual hydrogen atoms have been replaced by amino groups. Chemically, the majority of biological acids are α -amino acids, the $-\text{NH}_2$ group being attached to the C atom next the terminal carboxyl group.

Type formula :



where *R*, the hydrocarbon residue, may represent aliphatic, aromatic or heterocyclic radicles.

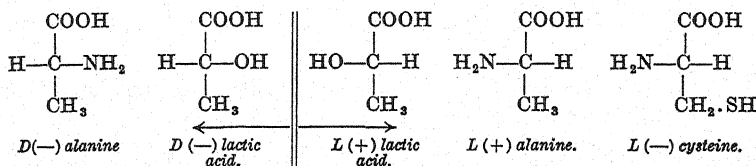
General Properties of the Amino Acids.—All the natural acids are colourless crystalline solids. All, except cystine, leucine, and tyrosine, are readily soluble in water; and all, except proline and hydroxyproline, are sparingly soluble in alcohol. All are optically active, except glycine, the α -carbon of which is not asymmetric. The dextro acids are: alanine, valine, iso-leucine, glutamic acid, hydroxyglutamic acid, aspartic acid, arginine, citrulline, and lysine. The others are levo-rotatory.

While amino acids may be classified as dextro (+) or levo (−) rotatory, a more logical plan has been worked out by Levene, who refers them to a parent molecule, tartaric acid, which exists in two optically isomeric forms, namely D-tartaric acid and L-tartaric acid, so called because they are spatially related to D-glucose and L-glucose, respectively. Actually D-tartaric acid is the levo-rotatory isomer, and to avoid confusion of notation, the two acids are written D (−) tartaric acid and L (+) tartaric acid.

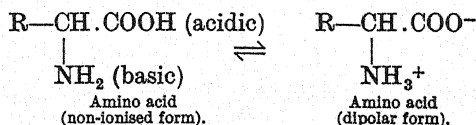
From L (+) tartaric acid is derived L (+) lactic acid, which is

also obtainable from the natural amino acid (+)alanine. Hence it is concluded that alanine is an L-acid, and a stereochemical descendant of L-glucose.

The common amino acids, irrespective of their optical rotation, belong to the L-series. A few members of the D-series have been found in micro-organisms.



Dipolar Form of the Amino Acids.—The monoamino-monocarboxy acids are neutral in solution, and are very weak electrolytes. At the same time they are able to neutralise either acids or bases. This property, termed *amphotericity*, is due to the presence of an acid and a basic group in the same molecule. In aqueous solutions amino acids ionise to form a dipolar or **zwitter-ion**, having two equal charges of opposite electric sign, and tending to migrate neither to anode nor cathode when a current is passed through the solution.

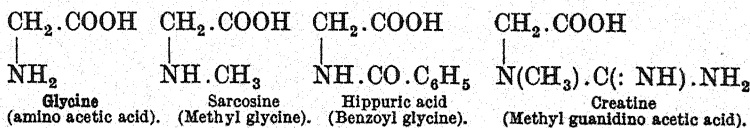


Acids are neutralised by the $-\text{COO}^-$ group of the amino acid which can combine with H^+ to form $-\text{COOH}$. Alkalies are neutralised by the $-\text{NH}_3^+$ group, which can donate H^+ by reverting to $-\text{NH}_2$.

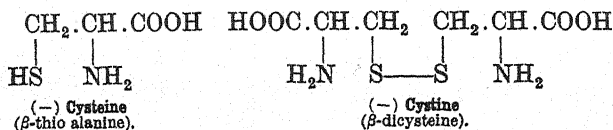
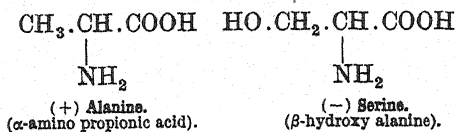
CLASSIFICATION OF THE AMINO ACIDS

The simplest classification refers each acid to the parent aliphatic acid.

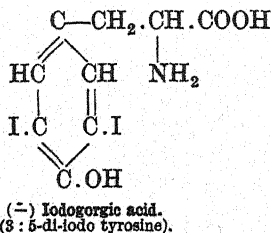
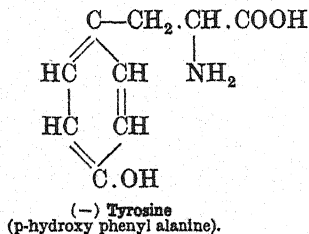
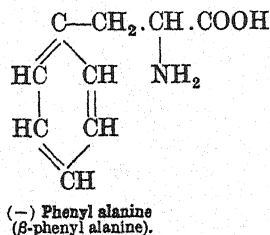
(1) **Acids derived from Acetic Acid : $\text{CH}_3\cdot\text{COOH}$.**



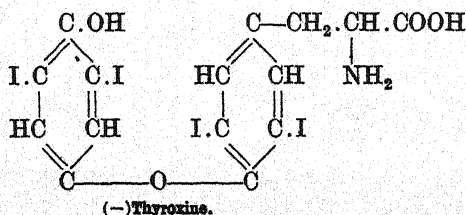
Glycine is the only one of these derivatives found as a protein unit. Sarcosine is a hydrolysis product of creatine. Hippuric acid and creatine are amino acid derivatives.

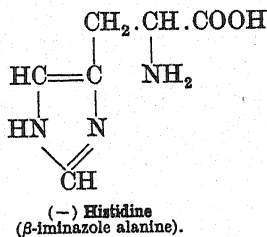
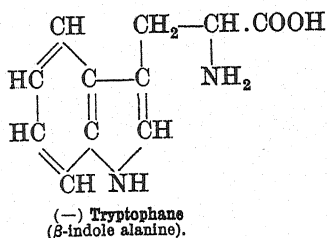
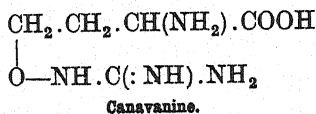
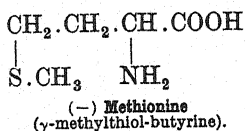
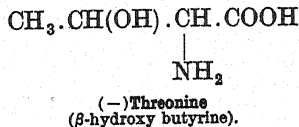
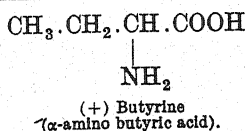
(2) Amino Acids derived from Propionic Acid : $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{COOH}$.

Cyclic Amino Acids derived from Alanine.—These may be regarded also as amino acid derivatives of aromatic and other nuclei.



Iodogorgic acid has only been found in the scleroprotein of sponges. It is of special interest on account of its chemical relationship to thyroxine, the characteristic amino acid of the thyroid gland.

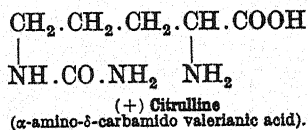
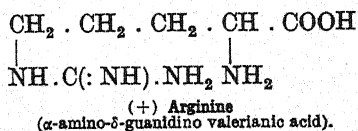
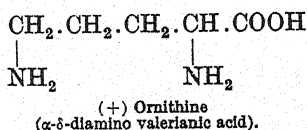
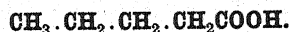


(3) Amino Acids derived from Butyric Acid: $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{COOH}$.

Methionine, like cystine, is a sulphur-containing amino acid, and a source of this element in animal nutrition.

Canavanine, isolated from Jack bean, resembles arginine in being a guanidine derivative and yielding urea on alkaline hydrolysis.

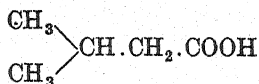
(4) Amino Acids derived from Valerianic Acid :



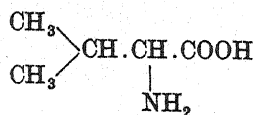
Arginine occurs in many proteins, and may be an essential part of the molecular pattern of all proteins; on hydrolysis it yields ornithine and urea.

Citrulline occurs free in the melon (*Citrullus*); and also as a protein constituent.

(5) Amino Acid derived from iso-Valerianic Acid.

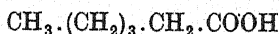


Iso-valerianic acid.

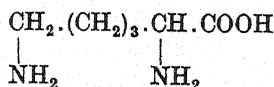


(+)-Valine
(α -amino iso-valerianic acid).

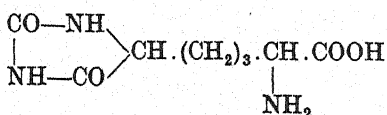
(6) Amino Acids derived from Caproic Acids.



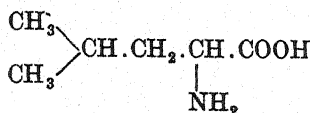
Caproic acid.



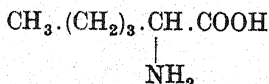
(+)-Lysine
(α - ϵ -diamino caproic acid)



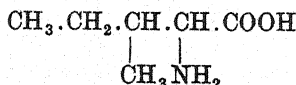
Prolyzine.



(-)-Leucine
(α -amino iso-caproic acid).

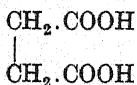


(+)-Nor-leucine
(α -amino caproic acid).

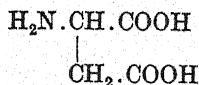


(+)-Iso-leucine
(γ -methyl-valine).

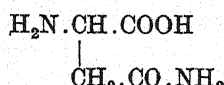
(7) Amino Acids and Amides derived from Succinic Acid.



Succinic acid.

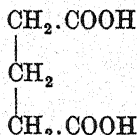


(-)-Aspartic acid.
(amino succinic acid).

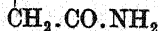


(-)-Asparagine
(β -amino succinamide).

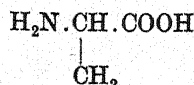
(8) Amino Acids and Amides derived from Glutaric Acid.



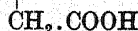
Glutaric acid.



(+)-Glutamine

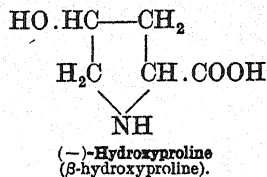
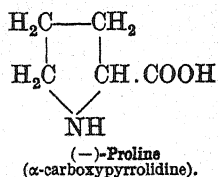
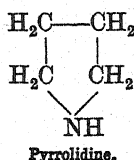


(+)-Glutamic acid
(α -amino glutaric acid).



(+)- β -hydroxy glutamic acid.

(9) Imino Acids derived from Pyrrolidine.



According to Wada (1933), *citrulline* is a precursor of proline, and *prolysine* is a precursor of lysine in protein hydrolysis.

Physiological Classification of Amino Acids.

(1) Essential for life of higher animals : *threonine, leucine, iso-leucine, phenylalanine, tyrosine, histidine, lysine, valine, methionine, tryptophane.*

(2) Non-essential. The remaining amino-acids, with the possible exception of *arginine*, or some unrecognised source of the guanidine nucleus required by growing animals. *Histidine* appears to be non-essential for the human subject.

(3) *Glucogenic* amino acids, capable of giving rise to glucose in the animal body.

(4) *Ketogenic* amino acids, giving rise to aceto-acetic acid when perfused through a surviving liver.

Fractionation of the Products of Protein Hydrolysis.—The amino acids can be separated analytically into three groups :—

(1) *The basic group*, precipitated by phosphotungstic acid. This group includes the three diamino acids : *arginine, histidine, lysine*, along with *cystine* and the imino acids, *proline* and *hydroxyproline*.

(2) *The acidic group*, precipitated as salts after addition of calcium hydroxide and alcohol up to 80 per cent. The group consists of the three dicarboxy acids, *aspartic, glutamic, and hydroxyglutamic*.

(3) *The neutral group* of monocarboxy-monoamino acids. No complete qualitative method of separation is known. On concentration, *tyrosine, leucine, and cystine*, the least soluble of the acids, crystallise out.

Cystine crystallises in characteristic hexagonal plates that are seen in urine and in urinary calculi in the rare condition of *cystinuria*. On reduction *cystine* is converted into two molecules of *cysteine*, which is readily soluble in water.

Tyrosine crystallises in tufts of fine, silky needles.

Leucine, which is usually closely associated with *tyrosine*, forms pearly plates and "cones."

The further separation of the acids requires special methods for each species. Thus, *arginine* is isolated as a salt of flavianic acid, *histidine* as a silver derivative, *tryptophane* as a mercury derivative.

Essential in diet.	Glucogenic.	Ketogenic.	Amino Acid.	Character.						
—	()	()	Citrulline.	Basic amino acids. 1 carboxyl and more than 1 basic group.						
—	()	()	Canavanine.							
+P	+	—	Arginine.							
+P	—	+?	Histidine.							
+	—	—	Lysine.							
—	P	P	Thyroxine.	Contain iodine.	Contain benzene ring.	Aromatic amino acids.	Neutral amino acids.			
—	P	P	Iodogorgic acid.							
P	—	+?	Tyrosine.	Phenolic.	Contain pyrrolidine ring.		1 carboxyl and 1 amino group.			
+	—	+?	Phenylalanine.							
+	—	—	Tryptophane.	Indolic.						
—	+	—	Proline.	Imino acids.						
—	+	—	Hydroxyproline.							
—	+	—	Cystine.	Contain sulphur.	Aliphatic acids.					
+	()	()	Methionine.							
+	—	+	Leucine.							
+	—	—	Iso-leucine.							
+	—	—	Valine.							
+	()	()	Threonine.	Hydroxy acids.						
—	+	—	Serine.							
—	+	—	Alanine.							
—	+	—	Glycine.							
—	+	—	Glutamic acid.	Acidic amino acids. 1 amino and 2 carboxyl groups.						
—	+	—	Hydroxyglutamic acid.							
—	+	—	Aspartic acid.							

() Indicates that data are not available.

Summary of the Amino Acids

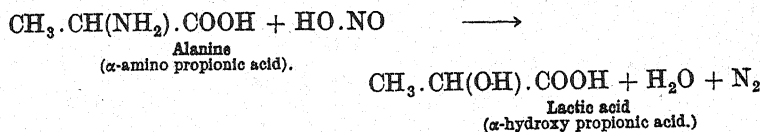
In the dicarboxyl acids the presence of a second carboxyl group dominates the single amino group, and for this reason aspartic, glutamic, and hydroxyglutamic acids have an acidic reaction in aqueous solution. The diamino acids, with the exception of citrulline, are strongly basic, owing to the presence of two amino groups and only one carboxyl.

General Reactions of Amino Acids.—The α -amino group can be removed by *deamination*, or destroyed by condensation with aldehydes or quinones; the carboxyl group can be removed by

decarboxylation. These reactions are common to all free amino acids, and are the basis of general methods for their estimation.

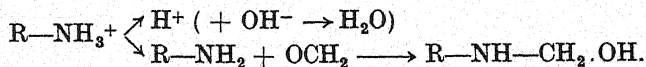
(1) *Deamination by Nitrous Acid.*—Free nitrous acid, $\text{HO}\cdot\text{NO}$, but not the nitrite ion, ONO^- , combines with free amino groups to form an unstable *diazo* compound, $\text{R}\text{—NH}\text{—NO}$, that hydrolyses spontaneously into $\text{R}\cdot\text{OH}$, H_2O and N_2 , which escapes as a gas, and can be measured.

When an amino acid is deaminated in this way the corresponding hydroxy acid is produced :—



In Van Slyke's method, the amino compound is added to a mixture of 30 per cent. sodium nitrite and glacial acetic acid contained in a special type of apparatus, which is shaken vigorously for five minutes. The evolved gas is expelled, treated with alkaline permanganate to absorb nitric oxide, which forms as a by-product, and the residual nitrogen is estimated in a manometer. Under standard conditions, the α -amino groups lose all their nitrogen within five minutes.

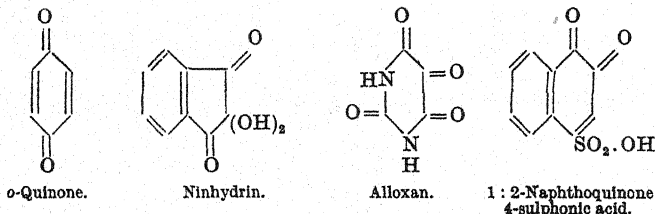
(2) *Formaldehyde Condensation.*—When an amino acid in neutral solution is mixed with excess of neutral formaldehyde solution, the mixture becomes acid, and can be titrated (Sørensen's reaction), thus affording an important method for the estimation of amino acids and ammonium salts. The mechanism of the change has been studied by Levy (1937). By the action of the alkali during titration, the ionised amine groups are changed from the protected form, —NH_3^+ , into the free form, —NH_2 , by removal of H^+ , which is neutralised by the OH^- of the alkali. The free amino group rapidly combines with the formaldehyde to produce a substituted amino alcohol, $\text{R}\text{—NH}\text{—CH}_2\cdot\text{OH}$, which is neutral to the indicator. Thus, the amount of alkali used is a measure of the amino groups originally present in the mixture.



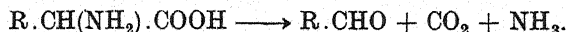
Proteins treated with formaldehyde lose the properties associated with their terminal amino groups, and by this means diphtheria and similar bacterial toxins can be converted into harmless *toxoids*, or *anatoxins*, which, however, still retain the power of acting as antigens when injected into animals, and thus are of value in conferring immunity.

(3) *Miscellaneous Amino Reactions*.—Phenyl isocyanate, $C_6H_5.N:CO$, even at $0^\circ C.$, reacts readily with free amino groups to yield substituted ureas of low solubility and easy identification, $C_6H_5.NH.CO.NH.R$.

Quinones and cyclic ketones, such as ninhydrin and alloxan, condense with amino compounds in alkaline or slightly acid solution to yield pigments, some of which are suitable for colorimetric estimation.

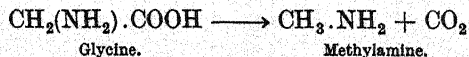


The Ninhydrin Reaction.—In 1911, Ruhemann showed that ninhydrin (triketo-hydrindine hydrate) quantitatively decarboxylated certain amino acids, with formation of CO_2 , NH_3 , and an aldehyde:

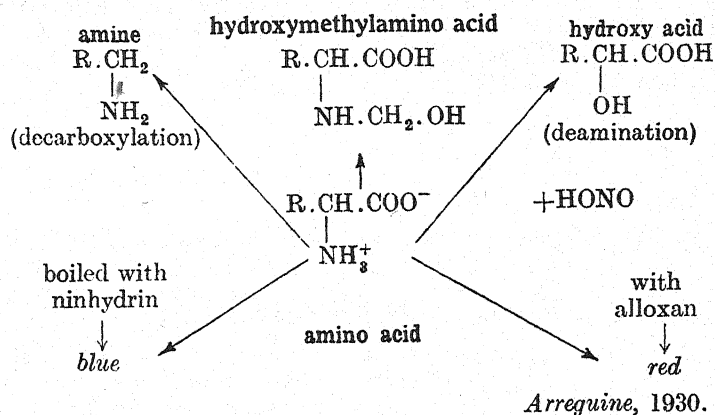


If the reaction occurs at a pH more alkaline than 4, the NH_3 combines with the excess reagent to give a blue pigment. The imino acids, proline and hydroxyproline, react to yield a red pigment, and evolve CO_2 but not NH_3 . Protein hydrolysates give various shades of violet, owing to the presence of both α -amino and imino groups, while ammonium salts and some amines yield blue colours. Hence the reaction is not sufficiently precise for general colorimetry. In 1938, however, Van Slyke and Dillon reported that the liberation of CO_2 was restricted to free α -amino acids, in that it required the presence of both $-COOH$ and an adjacent $-NH_2$ or $-NH-CH_2-$ group. When such compounds are boiled with water and excess of ninhydrin, at pH 1 to 5, all the CO_2 of the carboxyl groups is evolved in a few minutes, and can be measured volumetrically or titrated after absorption by alkali. By such means, Van Slyke and his colleagues (1941) have developed a rapid and selective method for the estimation of amino acids.

(4) *Decarboxylation*.—In addition to the ninhydrin reaction, amino acids can be decarboxylated by enzyme systems present in many micro-organisms, with production of the corresponding amine.



SUMMARY OF AMINO ACID REACTIONS



Amino Acid Percentage Content of Typical Proteins

Amino Acid.	Gelatin.	Casein.	Milk Albumin.	Egg Albumin.	Glutin.	Zein.	Edestin.
Glycine	25.2	0.4	0.4	1.7	0.0	0.0	3.8
Alanine	8.7	1.8	2.4	2.2	2.0	9.8	3.6
Valine	0.0	7.9	3.3	2.5	3.3	1.9	+
Leucine + Iso-leucine	7.1	9.7	14.0	10.7	6.6	25.0	20.9
Aspartic acid	3.4	4.1	9.3	6.2	0.8	1.8	10.2
Glutamic acid	5.8	21.8	12.9	13.3	43.7	31.3	19.2
Hydroxy glutamic acid	0.0	10.5	10.0	1.4	2.4	2.5	—
Serine	0.4	0.5	1.8	—	0.1	1.0	0.3
Proline	9.5	8.0	3.8	4.2	13.2	9.0	4.1
Hydroxyproline	14.1	0.3	—	—	—	—	2.0
Phenyl alanine	1.4	3.9	1.2	5.1	2.3	7.6	3.1
Tyrosine	0.01	6.5	1.9	3.2	3.1	5.9	4.5
Cystine	0.1	0.3	4.0	1.3	2.4	0.8	1.0
Arginine	9.1	5.2	3.0	6.0	3.2	1.8	15.8
Histidine	0.9	2.6	1.5	1.4	2.1	1.2	2.1
Lysine	5.9	7.6	8.4	3.8	0.6	0.0	2.2
Tryptophane	0.0	2.2	2.7	1.3	0.8	0.17	1.5
Methionine	1.0	3.6	2.7	5.2	2.0	2.9	2.6
Total	93	96.8	84.3	67.1	88.6	102.7	97

Values enclosed in a square denote that the protein is characterised by a high content of that particular acid.

When completely hydrolysed, 100 gm. of protein should yield about 120 gm. of mixed amino acids, owing to the water taken up.

Many of these amines, notably *tyramine*, from tyrosine, and *histamine* from histidine, are much more physiologically active than the parent amino acid, and their liberation is an important factor in surgical shock and similar conditions.

(5) *Racemization*.—With the exception of glycine, which has no asymmetric carbon, all the natural amino acids are optically active, and, with some curious exceptions, belong to the L-series, descending from L-lactic acid. Similarly, the proteins assembled from these amino acids are all more or less optically active.

When a protein or simple peptide is kept in alkaline solution, it gradually loses its optical activity, owing to the constituent amino acid units undergoing *racemization*, or conversion into an equilibrium mixture of optical isomers. Since free amino acids are not easily racemised by alkalis, Dakin has ascribed the change to *cis-trans* rotation at the peptide linkages, which become double-bonded in alkaline solution. After racemization, proteins are no longer hydrolysed with the same facility by enzymes, showing that there is biological preference for particular optical configurations.

The Molecular Weight and Dimensions of the Protein Molecule.—Proteins and their cleavage products down to the smaller polypeptides form colloidal solutions in water; this implies that they are compounds of much greater molecular size and weight than, say, the simple sugars and the amino acids, and the ordinary thermal methods used for determining molecular weights are inapplicable, since colloids undergo changes when boiled or frozen in solution. The methods employed are (i.) *stoichiometric*, according to which the molecular weight is deduced from the content of a particular amino acid or element such as iodine, sulphur or iron, present in the molecule, on the assumption that the protein contains a simple multiple of the unit; (ii.) *physical*, depending on measurements of osmotic pressure, sedimentation equilibrium and sedimentation rate, using an ultra-centrifuge.

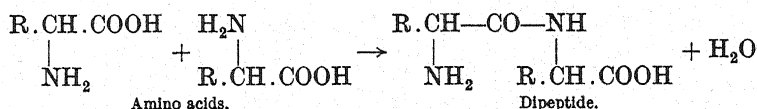
Sedimentation data obtained by Svedberg indicate that proteins fall into two classes: (i.) those with a radius between $2\ \mu$ and $4\ \mu$, and a molecular weight that is a simple multiple of 35,000; and (ii.) those with a radius of $12\ \mu$, and upwards, and a molecular weight that is approximately a multiple of 400,000.

Examples of class (i.) are: lactoglobulin (37,800), pepsin (35,000), insulin (41,000), ovalbumin (40,500), hæmoglobin (68,000), serum globulin (150,000). Examples of class (ii.) are the hæmocyanins, one of which in the snail has a value of about 5,000,000.

By X-ray analysis, Astbury and his colleagues have shown that proteins are composed of repeating structural units, the length and position of which may be measured. These observations

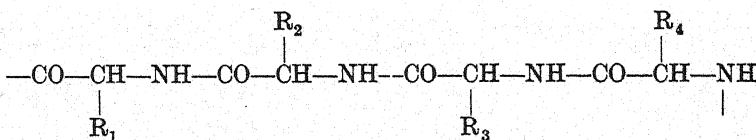
explain the distinction recognised between *fibrous* non-crystalline proteins, composing silk, hair, muscular and connective tissue, and *globular* crystalline proteins, such as egg albumin, hæmoglobin and insulin.

Structure of the Protein Molecule.—Since proteins are assembled from amino acid units, the simplest structure they can have is that of a polypeptide chain, which may be fragmented into smaller peptides. A dipeptide is a compound formed by condensation between the carboxyl group of an amino acid with the amino group of another acid. A tripeptide is formed by addition of an amino acid to a dipeptide.



Glycyl-glycine, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CO} \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{COOH}$, is the simplest peptide.

(1) **The Peptide Theory** (Hofmeister and Emil Fischer).—The protein molecule is a chain composed of peptide links, and has the type formula :—



R represents various monovalent groups, twenty-two of which are now known, which form side-chains to a common main-chain.

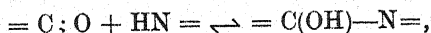
Evidence in Support of the Peptide Theory.—(i.) All proteins give a violet or rose colour with copper salts in alkaline solution (the copper protein test), which is characteristic of the peptide linkage $=\text{CH}-\text{CO}-\text{NH}-\text{CH}=$, and not given by any individual amino acid other than serine, threonine and histidine, which have a somewhat similar arrangement in their molecules.

(ii.) During protein hydrolysis there is little change in the reaction. The basic amino and the acid carboxyl groups are exposed at an equal rate, showing that they were originally present in combination.

(iii.) Natural proteins, other than protamines and histones, are neutral compounds, and the free amino-groups that they display have been shown to be the terminal groups of lysine or arginine.

(iv.) Peptides of known constitution have been synthesised, some of which are hydrolysed by the proteoclastic enzymes in a manner comparable to the hydrolysis of natural proteins.

Developments of the Peptide Theory.—(a) *Lattice Structure.*—When a crystal is irradiated by X-rays, secondary rays are reflected from each surface within the structure, and form interference patterns that can be photographed. From a study of these patterns, Meyer, Astbury and others have concluded that the typical ground plan of the protein is a crinkled ribbon of peptide main-chains, with side-chains projecting alternately upwards or downwards from the edges of the ribbon. The main-chains are held in bundles by cross-union between neighbouring keto and imino groups :

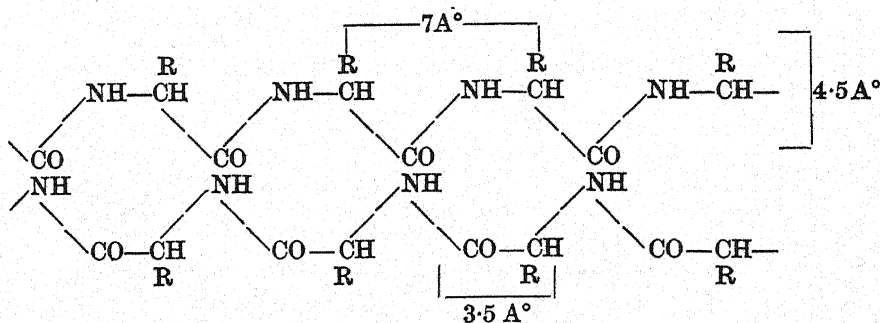
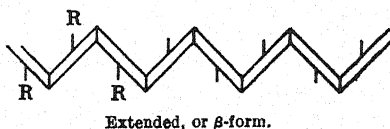
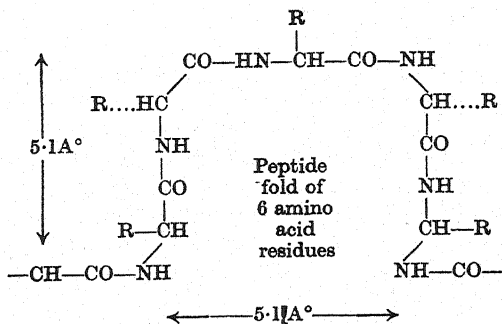
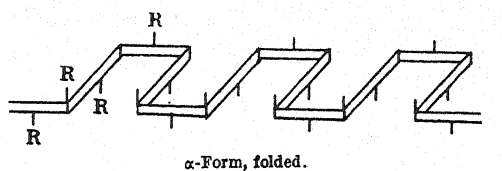


thus forming a grid or lattice.

According to Astbury (1941), two extreme types of protein structure exist : (1) visibly fibrous but not visibly crystalline, such as hair, silk and tendon ; and (2) visibly crystalline but not fibrous, such as egg albumin, hæmoglobin and insulin. The distinction is not always sharp. The densities of all proteins, whether fibrous or non-fibrous, is similar, being about 1.3. Radiograph patterns of non-fibrous proteins usually show an inner halo, due to reflection from the side-chains, and a diffuse outer halo, due to main-chain " back-bone " reflection. The patterns of fibrous proteins show the existence of an α -form, as in keratin and myosin, in which the main-chain is closely folded, and the fibres are elastic ; and the existence of a β -form, as in silk and collagen, in which the main-chain, though crinkled, is not closely folded, and the fibres are relatively inelastic. When stretched, proteins of the α -form acquire the X-ray pattern characteristic of the β -form.

Chemical analysis of wool keratin, a protein of m.w. 68,000, shows that each unit has 576 amino acid residues, represented by : glycine, 64 ; glutamic acid, 64 ; leucine and *iso*-leucine, 64 ; cystine, 72 ; serine, 64 ; arginine, 36 ; proline, 36 ; alanine, 32 ; threonine, 32 ; aspartic acid, 32 ; valine, 24 ; phenylalanine, 16 ; tyrosine, 16 ; lysine, 12 ; tryptophane, 6 ; histidine, 3 ; methionine, 3.

Any structural plan for a protein of the α -form, such as wool keratin, must, as an extensible polypeptide, allow room for the various amino acid residues within the space limits indicated by the X-ray pattern. Consequently, Astbury has suggested a structure in which R, the amino acid residues, are in *cis-trans* arrangement, projecting alternately upwards or downwards from the edges of the ribbon-like main-chain.

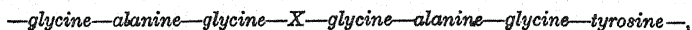


The average thickness of an amino acid residue in the chain is about 4.5 Å (0.45 mμ), and its length is about 3.5 Å.

Hair is characterised by the appearance of a new type of X-ray pattern when it is stretched, showing that the keratin chains occur normally in a folded state. Hair protein resembles muscle protein that has been "vulcanised," or impregnated with sulphur, to decrease plasticity and increase resistance to wear.

(b) *Pattern Frequency*.—From a study of the amino acid ratios in proteins, and the composition of derived peptides, Bergmann

and Niemann conclude that the amino residues are arranged in regular order and frequency in the molecule. For example, silk fibroin has the pattern :—



where X represents an unidentified unit.

In egg albumin, the amino acids occur in the following frequencies : glutamic acid, 1 in 8 ; aspartic acid, 1 in 18 ; methionine, lysine and arginine, 1 in 24 ; tyrosine, 1 in 36 ; histidine and cysteine, 1 in 72. The smallest number containing these ratios is 288, hence the molecular weight of egg albumin must be some multiple of 288. A molecule of 288 residues has a molecular weight of 288 multiplied by the average weight of the residues, which gives a value of 35,700, a result of the same order as that obtained for egg albumin (40,500) by means of Svedberg's ultracentrifuge.

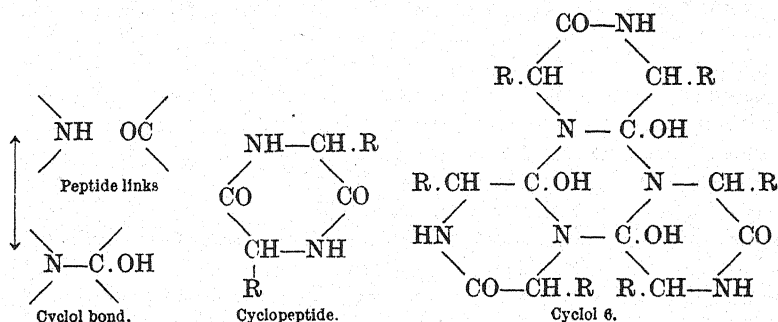
According to the requirements of pattern frequency, proteins must be assembled from aggregates of $2^m \times 3^n$ units, where m and n are whole, positive numbers.

The frequency hypothesis has been critically reviewed by Chibnall (1942), who points out that confirmation can only be obtained when full quantitative data are available, especially for the basic and dicarboxy acid residues. These have now been got for at least three proteins : *edestin*, the smallest possible unit of which has 432 residues ; β -lactoglobulin, a homogeneous protein of m.w. 42,000, with a system of 9 peptide chains, not all of like composition ; and egg albumin, m.w. 43,000, a non-homogeneous protein, with a molecular system of at least 4 peptide chains having free terminal groups. Chibnall concludes that the Bergmann-Niemann hypothesis is only applicable to the patterns of the individual peptide chains in the entire protein molecule.

(2) **The Cyclol Theory.**—Protein molecules are composed largely, if not entirely, of amino acid residues, and contain many —NH—CO— linkages but few free —NH₂ groups, apart from those found in side-chains. The general uniformity of the protein type suggests a uniformity of general structure. Many proteins are easily denatured, and when spread on a suitable surface spontaneously form insoluble monolayers.

Globular proteins in alkaline solution tend to aggregate. Protein crystals show a high trigonal symmetry. These and other observations have led Wrinch (1936) to discard the peptide chain theory in favour of a structure in which the unit is a cyclol made up of six amino acid residues. Each cyclol is further stabilised by cross

union between a peptide nitrogen and an adjacent carbon atom, the result being a "cyclol 6," which reoccurs throughout the protein fabric.



By folding a sufficient number of cyclols it is possible to make a closed cage, which according to Wrinch, represents the protein molecule.

These cages are in the form of hollow truncated tetrahedra, constructed from a simple multiple of 72 amino acid units, the R radicles of which either project into the cavity or extend outwards from the surfaces. Wrinch suggests that the globular molecules of insulin, pepsin and egg albumin contain 72×4 amino acid units, which conforms with Svedberg's data for dimension and density, and with the conclusions of Bergmann, but awaits confirmation by X-ray analysis. The cyclol theory has been supported by Langmuir, and criticised by Astbury (1941) and Neurath (1942) on the grounds that the space enclosed in the cyclol cage is insufficient to accommodate all the necessary amino acid side-chains. In her later developments of the theory, Wrinch has offered an explanation of the surface properties of the protein molecule, especially in reference to cell membranes and cell organisation (1942).

Titration Curves for Amino Acids and Proteins.—When a solute is titrated by an acid or a base, and the resulting changes in pH are plotted for each unit of acid or alkali added, a titration curve is obtained, showing the buffering power of the solute. With water or solutions of neutral salts, such as NaCl , the pH change on either side of neutrality is abrupt; with amino acids or proteins, the change is gradual, owing to the acid-buffering power of the $-\text{COO}^-$ groups, which combine with H^+ , and the base-buffering power of the $-\text{NH}_3^+$ groups, which release H^+ . The quantitative character of this reaction was first studied by Loeb (1922), who ascribed it to the

"acid-binding" and "base-binding" properties of the protein, in conformity with the early concept of salt-formation in solution by union between —NH_2 and acids, or —COOH and bases. The present explanation, which is in conformity with the modern theory of solutions, is founded on the work of Harris (1925), who showed that when the free amino groups of an amino acid or a protein are masked by combination with formaldehyde, the solute still retains its power for neutralising acids, showing that this property resides in the ionised —COO^- groups and not in the —NH_2 groups. At a particular pH, all the —NH_2 groups of a protein in solution are in the acidic, or base-neutralising, form of —NH_3^+ , and all the —COOH groups are in the basic, or acid-neutralising, form of —COO^- . The protein molecule, hence, is electrically neutral, as a balanced dipolar ion, and migrates neither to the anode nor the cathode when a potential difference is set up in the solution by means of electrodes. In this condition, which is determined by the pH, the protein is said to be at its *iso-electric point*.

ANALYTICAL REACTIONS OF PROTEINS

A. General Colour Reactions given by all Proteins.—These depend on characteristic amino acid groupings in the protein molecule.

(1) **The Protein Copper Test, or "Biuret" Test.**—To 5 ml. of solution add 1 ml. of 20 per cent. NaOH. Then add 1 or 2 drops of dilute (1 per cent.) CuSO_4 . If a protein be present the solution becomes purple. Using the minimal quantity of copper sulphate, it is possible to distinguish two shades of colour :—

(i.) *Violet*, given by all higher proteins and also by gelatin.

(ii.) *Rose-pink*, given by all lower proteins (proteoses, peptones, peptides), except gelatin. Excess of copper renders these shades indistinguishable.

Acidify the mixture with 20 per cent. acetic acid until the colour is discharged. A precipitate forms if the solution contains a higher protein, not if it contains a lower protein.

The colour depends on the presence of the peptide linkage —CH—CO—NH—CH= in the protein molecule. A somewhat similar colour is given by other compounds containing this grouping, such as *biuret*, $\text{H}_2\text{N—CO—NH—CO—NH}_2$, hence the older and misleading name for the test. Proteins do not contain biuret.

Acidification is of use in distinguishing between a doubtful violet or rose colour. Proteins (except gelatin) that give a violet copper reaction are precipitated on subsequent acidification of the mixture. No protein giving a rose colour is precipitated on subsequent acidification.

Ammonium salts inhibit the test by forming a deep blue colour with the CuSO_4 ; this may be overcome by addition of a large excess of NaOH, but it is a common source of error in testing filtrates after saturation with ammonium sulphate.

(2) **The Arginine Test.**—Make 5 ml. of solution alkaline with 5 drops of 20 per cent. NaOH. Add 5 drops of 2 per cent. alcoholic α -naphthol, followed by 5–10 drops of 2 per cent. sodium hypochlorite (NaOCl) or 1–2 drops of bromine water. An intense carmine develops if arginine or a protein containing arginine be present. Arginine appears to be a universal constituent of proteins, and the reaction may be regarded as a general protein test.

The colour depends on the presence of the monosubstituted *guanidine grouping*, $\text{H}_2\text{N}-\text{C}(\text{:NH})-\text{NH}\cdot\text{R}$, in the molecule.

(3) **Ninhydrin**, the general reagent for amino acids (p. 156), also reacts with proteins in slightly alkaline solution to give violet colours.

B. Colour Tests due to Individual Amino Acids

(1) **Xanthoprotein Test.**—Add 5 drops of concentrated nitric acid to about 3–5 ml. of protein solution. A precipitate forms if higher proteins be present (Heller's test). Heat the mixture. A yellow colour develops if the test be positive. Cool, and make alkaline with ammonium hydroxide. The colour deepens to orange.

The test is due to the presence of substituted benzene rings occurring as tyrosine and tryptophane in the protein. It is given also by free tyrosine, tryptophane and other benzene derivatives.

Gelatin is the commonest protein that does not give the xanthoprotein test owing to its lack of the necessary amino acids.

(2) **Tyrosine Test.**—Add 5 drops of 9 per cent. mercuric sulphate in 10 per cent. sulphuric acid to 5 ml. of protein solution. A precipitate forms with most proteins. Heat the mixture and add a drop of 1 per cent. sodium nitrite. A red colour develops if tyrosine be present.

This is Cole's modification of the original test, in which Millon's reagent is used, unaccompanied by sodium nitrite.

The test is given by many phenolic compounds, of which tyrosine is the only one found in proteins.

Chlorides and excess of alkali inhibit the test by precipitating the mercury. This can be compensated by addition of more reagent.

Pure gelatin with a tyrosine content of about 0.01 per cent. gives a negative result with the test when in solutions of less than 20 per cent. concentration.

(3) **Tryptophane Tests.**—(i.) Add 3 drops of Ehrlich's aldehyde reagent to about 1 ml. of protein solution, followed by excess (3–5 ml.) of concentrated hydrochloric acid. Heat gently to boiling. A violet colour develops if the protein contains tryptophane. Cool and add 1–3 drops of 0.1 per cent. H_2O_2 . The colour changes to deep blue. Excess of the oxidiser rapidly bleaches the pigment.

This test is very sensitive, and will detect 1 part of tryptophane in 500,000.

The reagent is 2 per cent. *p*-dimethylamino-benzaldehyde in alcohol or 5 per cent. hydrochloric acid. It gives characteristic colours with sulphonamides, indole and scatole (p. 352), urobilinogen (p. 206), urea (p. 440), and allantoin (p. 450). Tryptophane appears to be the only biological compound that gives a deep blue colour with the reagent.

(ii.) Mix 3 ml. of solution with about 2 ml. of the Hopkins-Cole reagent or with 2 ml. of glacial acetic acid that has been "activated" by exposure to sunlight. Then add slowly about 3 ml. of concentrated sulphuric acid so as to form a layer below. A deep purple ring develops at the junction of the liquids if tryptophane be present.

The active ingredient in both reagent and acetic acid is glyoxylic acid, $\text{HOOC}\cdot\text{CHO}$, the recognition of which led to the subsequent discovery of tryptophane by Hopkins and Cole.

Most proteins, with the exception of gelatin and zein, give a positive result with the tryptophane tests.

(4) **Cystine Test.**—Add a few drops of 5 per cent. lead acetate to 5 ml. of protein solution, followed by sufficient 20 per cent. sodium hydroxide to redissolve the white precipitate of lead hydroxide that first forms. Boil the mixture. If cystine or proteins containing cystine be present, the solution turns dark brown owing to formation of lead sulphide.

(5) **Thiol Test.**—Mix equal volumes (2–3 ml.) of protein solution and saturated ammonium sulphate. Add 2–4 drops of fresh 5 per cent. sodium nitroprusside, and make alkaline with a few drops of ammonium hydroxide. If metaprotein, glutathione, cysteine, or other compounds containing the thiol group, $-\text{SH}$, be present, a purple colour develops.

Heat-coagulation or acid-denaturation of higher proteins unmasks thiol groups. During subsequent hydrolysis these groups tend to disappear, perhaps by oxidation to dithio groups, $-\text{S}-\text{S}-$, such as occur in cystine, which does not give the test.

A somewhat similar colour reaction is given by acetone and acetic acid (Rothera's test, p. 463).

(6) **Citrulline Test.**—Add 3 drops of 3 per cent. diacetyl monoxime to 2 ml. protein solution. Add about 3 ml. of concentrated hydrochloric acid, or sufficient to redissolve any protein that may be precipitated. Heat to boiling for half a minute. Allow to cool, and carefully oxidise by addition of 1–2 drops of very dilute (0.1 per cent.) hydrogen peroxide or 1 per cent. potassium persulphate.

If the test be positive a carmine colour gradually develops, and may be intensified by heating. Excess of oxidiser rapidly bleaches the pigment.

The test is given by mono-substituted ureas, of which citrulline is the only example known to occur in ordinary proteins.

Prolonged boiling must be avoided. Otherwise, extraneous colour reactions may arise from carbohydrate and tryptophane present in many proteins. Urea gives a bright yellow with the reagent.

In alkaline solutions, free diacetyl reacts with arginine and with creatine, and other substituted guanidines to give red pigments (Harden's test).

Group Tests for Proteins

(1) Heat-Coagulation (Albumins and Globulins)

Add a few drops of "Universal" or other appropriate indicator to 5 ml. of the protein solution, and adjust to the isoelectric region, pH 5-6, either by dilute acetic acid or sodium carbonate. Add a drop of 5-10 per cent. sodium chloride. Boil gently. A white coagulum shows the presence of albumin or globulin.

If metaproteins are present, they will be precipitated at pH 5-6 in the cold, and must then be removed by filtration before continuing the heat-coagulation test.

(2) Fractional Precipitation by Neutral Salts

(a) *Half-saturation*.—Mix 5 ml. of solution with an equal volume or slightly more, of saturated ammonium sulphate.

A white precipitate is given by globulins, caseinogen and other higher proteins, with the notable exception of albumins.

(b) *Full-saturation*.—Filter the mixture after half-saturation and add 5 ml. of the filtrate to a test tube containing about one-third its volume of solid ammonium sulphate. Mix well. All higher proteins and lower proteins, except peptones and peptides, are precipitated, as is shown by the appearance of a turbidity in the liquid above the layer of undissolved ammonium sulphate.

Fractional precipitation, or "salting-out," is used in the separation and identification of mixed proteins. Unlike heat-coagulation, it is a reversible process, and the protein precipitate can be redissolved on addition of water or removal of the salt by dialysis.

MgSO₄, Na₂SO₄ and NaCl are also used as neutral precipitants but are not as effective as (NH₄)₂SO₄ or ZnSO₄.

(3) Precipitation by Special Reagents.

Acids, Metals, and Alkaloidal Reagents.—Concentrated strong acids, notably nitric and trichloroacetic, CCl₃.COOH, precipitate most proteins higher than peptones; concentrated tannic acid precipitates peptones as well as higher proteins.

Heller's Test, which is important in the detection of albumin in urine, is carried out by placing about 2 ml. of concentrated nitric acid in a test tube, and carefully adding about 5 ml. of solution so as to form a layer on top. If a higher protein be present a white, cloudy ring forms at the junction of the liquids.

The Salicylsulphonic Acid Test.—A few drops of a 20 per cent. solution of this acid will give a white precipitate with higher proteins even in very dilute solutions. Peptones are not precipitated.

Proteins, especially in acid solution, are precipitated by many of the "heavy metals," including silver, mercury, lead, and copper. For this reason, egg-white is given as an antidote in poisoning by these metals.

ESTIMATION OF PROTEINS

(1) **Total Nitrogen Determinations.**—Since proteins are the outstanding nitrogen compounds of the organism they are often estimated in tissues and precipitates by determining the total nitrogen, and multiplying the result by a factor, usually 6.5, since proteins contain about 15–16 per cent. of nitrogen. The result gives the protein percentage in the material.

The total nitrogen is found by Kjeldahl's method, in which a known weight of material is boiled with concentrated sulphuric acid until all its nitrogen has been fixed as ammonium sulphate. Excess of sodium hydroxide is then added to liberate the ammonia, which is distilled over into a known volume of standard acid. From the amount of acid neutralised, the volume of liberated ammonia can be calculated and expressed in terms of nitrogen.

(2) **Amino Nitrogen Determinations.**—In this method, developed by Van Slyke, the mixture is treated with nitrous acid in a special apparatus. Each molecule of nitrogen gas set free corresponds to one molecule of nitrous acid and one α -amino group, and from this the total number of such groups present is calculated.

(3) **Decarboxylation** occurs when free amino acids are boiled with ninhydrin, and the CO_2 evolved can be measured. Van Slyke and Dillon (1938) regard this as the most specific method for estimating amino acids.

These methods are adapted for following the progress of protein hydrolysis, and for estimating the protein and amino acid content of the various fractions.

(4) **Colorimetric Methods.**—Many of the protein colour tests can be made quantitative by comparing the intensity of the colour with a standard test containing a known amount of the same protein.

(5) **Precipitation and Coagulation Methods.**—Albumin and globulin can be estimated absolutely by weighing the dry residue after heat coagulation and filtration. They and other higher proteins can also be estimated approximately by picric acid precipitation (Esbach's method), the volume of the precipitate being read after the mixture has stood for twelve hours in a graduated tube.

The cloudy precipitate formed on addition of salicylsulphonic acid may be matched in a comparator against a series of standard precipitates. This method is now employed in the estimation of protein in urine; it is an example of *nephelometry* or measurement of opalescence.

Percentage Distribution of Protein in Natural Sources

- Blood, human : Total protein, 18–21 ; hæmoglobin, 13–16.
 Plasma, human : Total protein, 7–8 ; seralbumin, 4–6 ; serglobulin, 1–2·3 ; fibrinogen, 0·3–0·6.
 Milk, human : Total protein, 1–1·6 ; caseinogen, 0·3 ; lactalbumin, 0·7–1.
 Milk, cow : Total protein, 2·6–3·5 ; caseinogen, 2·5–3 ; lactalbumin, 0·5.
 Muscle, human : Total protein, 17–19 ; myosin, 12–15 ; myogen, 4–6.
 Egg white, hen : Total protein, 10–13 ; ovalbumin, 9–11 ; ovoglobulin, 0·7 ; ovomucoid, 1.
 Egg yolk, hen : Total protein, 15–16 ; vitellin, 11–13 ; livetin, 3–4.

Summary of the Reactions of the Commoner Proteins

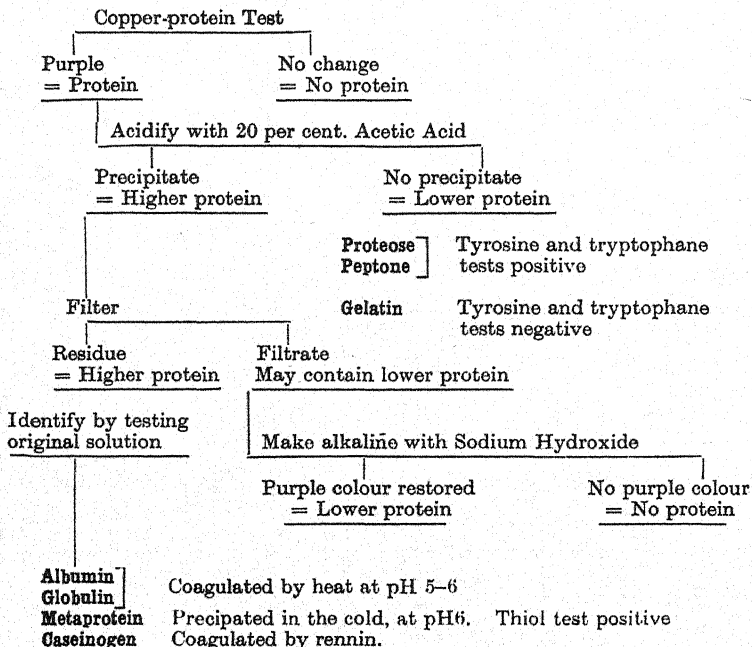
A. Colour Tests

Protein.	Copper Test.		Xantho-protein.	Tyrosine.	Tryptophane.	Arginine.	Citrulline.
		+ acid.					
Albumin .	violet	ppt.	+	+	+	+	+
Globulin .	violet	ppt.	+	+	+	+	+
Metaprotein .	violet	ppt.	+	+	+	+	+
Proteose .	rose	no ppt.	+	+	+	+	+
Peptone .	rose	no ppt.	+	+	+	+	+
Caseinogen .	violet	ppt.	+	+	+	+	+
Gelatin .	violet	no ppt.	—	—	—	+	(+)

B. Precipitation Tests

Protein.	Heat-coagulation.	Half-saturation with $(\text{NH}_4)_2\text{SO}_4$.	Full-saturation with $(\text{NH}_4)_2\text{SO}_4$.
Albumin . .	coagulates	not precipitated	precipitated
Globulin . .	coagulates	precipitated	precipitated
Metaprotein . .	precipitates at pH6, and then coagulates if heated at pH6.	precipitated	precipitated
Proteose . .	non-coagulable	not precipitated	precipitated
Peptone . .	non-coagulable	not precipitated	not precipitated
Gelatin . .	non-coagulable	precipitated	precipitated
Caseinogen . .	non-coagulable	precipitated	precipitated

DETECTION OF PROTEINS IN SIMPLE MIXTURES



This scheme of analysis is useful for the examination of artificial mixtures and peptic and tryptic digestion products. It does not differentiate between albumin and globulin or between proteose and peptone when present along with other higher and lower proteins. Such differentiation can be accomplished best by fractional precipitation with neutral salts, supplemented by the foregoing tests.

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CHAPTER 10

LIPIDES

LIVING organisms are not soluble in water ; they may be drowned, but they are not dissolved. Resistance is conferred partly by the use of protective coverings of scleroproteins on the surface of the animal body, and partly by the presence of water-insoluble compounds incorporated into the tissues and cell membranes. Many of these compounds are fats or fat-like substances, and can be extracted from dried animal and plant tissues by appropriate organic solvents. All these compounds are soluble in ether, and in this respect differ sharply from the carbohydrates and the proteins, and constitute the third great family of bio-organic compounds, namely, the **lipides**.

Definition.—Lipides are esters of higher aliphatic acids obtained from living tissues and characterised by insolubility in water, and solubility in the “ fat solvents ” : ether, chloroform, carbon tetrachloride, light petroleum (petrol), benzene, xylene, hot alcohol and hot acetone.

Distribution.—Lipides are universally distributed, partly as structural lipid or “ tissue fat ” in all cells, especially those of the nervous system, and partly as storage lipid, or “ reserve or depot fat ” in adipose tissue, seeds rich in oil, and other specialised regions. The distinction is physiological ; storage lipid is used in auto-nutrition, structural lipid is not.

Nomenclature.—Lipides may be divided into :—

<i>Simple Lipides.</i> (The fat group).	<i>Complex Lipides.</i> (The lipine group).
Contain only C, H, and O.	Contain C, H, O, N, and often P and S.
Mostly found as storage lipid.	Mostly found as structure lipid.
Usually simple glycerides of aliphatic acids.	Complex esters of various acids and bases.

In addition there are the *lipide derivatives*, products of hydrolysis, many of which are soluble in fat solvents.

There is an indefinite group of fat-soluble compounds, such as the carotene pigments, the terpenes, and the essential oils, which are removed along with the lipides during the process of extraction.

Although they are similar in regard to solubility they are quite distinct chemically and physiologically from either the fats or the lipines, and are excluded from the lipid family.

Classification of the Lipides.—A. *Simple lipides*, or *true fats*. Esters of higher aliphatic acids with various alcohols. Contain only C, H and O.

- (1) **Lipide Oils**, or *Oleo-lipides* ; glycerol esters liquid at 20° C.
- (2) **Fats** ; glycerol esters solid at 20° C.
- (3) **Waxes** ; esters of aliphatic acids and higher alcohols ; usually solid at ordinary temperature.

The term "oil" emphasises a physical property, and is applied to the true liquid fats and to at least two other groups of unrelated compounds : (1) the liquid paraffins, which are hydrocarbons and are not utilisable by animals or plants ; and (2) the "essential oils," which are volatile and odorous cyclic compounds of plant origin.

B. *Complex Lipides*, or *Lipines*.—Esters of aliphatic acids and complex alcohols. They contain C, H, O, N, and sometimes P and S.

- (1) **Phospholipides** (phospholipines or phosphatides) : Lipines containing phosphoric acid and a nitrogenous base.
Examples are : *lecith...*, *cephalin*, *sphingomyelin*.
- (1A) **Phosphotidates** : Phospholipides from which the nitrogen base has been removed.
- (2) **Glycolipides** (galactosides or cerebrosides) : Esters of aliphatic acids and carbohydrates. They contain N but no P.
Examples are : *kerasin* and *phrenosin*.
- (3) **Aminolipides** : compounds of aliphatic acids and substituted amines. Example : *bregenin*. They contain no P.
- (4) **Sulpholipides** : Lipides containing sulphuric acid and a nitrogenous base. Example : *sulphatide* from brain.

C. *Lipide Derivatives*.—Products of hydrolysis.

- (1) **Lipide Acids**.—Higher members of the acetic series of acids and related acids of the unsaturated oleic, linoleic, and linolenic series.
- (2) **Lipide Alcohols**.—Glycerol (glycerin) and higher solid alcohols.
- (3) Miscellaneous bases.

Lipoids.—These are biological compounds resembling the lipides in certain physical properties, notably solubility in fats and fat-solvent. The term is sometimes extended so as to include the complex lipides.

Lipoids as a type are non-saponifiable, although some of the sterols may occur as esters with aliphatic acids.

Significance.—Oleo-lipides and fats form a large group of natural compounds that occur in plants and in all terrestrial and marine animals, and provide an important class of food material. Chemically, they are *glycerides*, or esters of the tri-hydroxy alcohol glycerol and

three molecules of higher fatty acids, which are long, straight-chain members of an ascending series from C_{14} to C_{24} , increasing by $2C$ at a time. The glyceride structure of common fat was discovered by Chevreul, in 1823; since then, some 600 different natural fats have been recognised (Hilditch, 1939). Acids as low as C_{10} and as high as C_{30} occur in specialised lipid secretions.

A characteristic of natural fats is the predominance of fatty acids with an even number of total C atoms in the molecule, suggesting that fat-synthesis proceeds by addition of C_2 units to a C_{2n} nucleus.

LIPIDE DERIVATIVES

All fats, aliphatic oils, and waxes belong to the ester class of compounds, and on hydrolysis liberate alcohol and acids. Hence, in studying the lipides it is convenient to start with a survey of these aliphatic or lipidic acids.

Aliphatic Acids.—At least eight different series of organic acids are represented in the natural lipides. One of these, the acetic series, is saturated; the others are all more or less unsaturated.

(1) **The Acetic or Stearic Series, $C_nH_{2n+1}COOH$.** The chief members are:—

Acid.	Formula.	Solubility in H_2O .	Occurrence.
Formic	$H.COOH$	Miscible	Stings of insects and plants.
Acetic	$CH_3.COOH$	"	Vinegar.
Butyric	$C_3H_7.COOH$	"	Butter fat.
Caproic	$C_5H_{11}.COOH$	4 : 100 at $15^\circ C$.	Coconut oil.
Caprylic	$C_7H_{15}.COOH$	9 : 1000 at $15^\circ C$.	Palm oil.
Capric	$C_9H_{19}.COOH$	1 : 1000 at $100^\circ C$.	Laurel oil.
Myristic	$C_{13}H_{27}.COOH$	Insoluble	Nutmeg oil.
Palmitic	$C_{15}H_{31}.COOH$	"	Most animal and vegetable fats and oils.
Stearic	$C_{17}H_{35}.COOH$	"	Most animal and vegetable solid fats.
Arachidic	$C_{19}H_{39}.COOH$	"	Peanut (Arachis) oil.
Lignoceric	$C_{23}H_{47}.COOH$	"	Glycolipides.
Cerotic	$C_{25}H_{51}.COOH$	"	Beeswax.
Melissic	$C_{29}H_{59}.COOH$	"	Beeswax.

Tuberculo-stearic (10-methyl stearic), $C_{18}H_{37}.COOH$ and phthioic acid, $C_{25}H_{51}COOH$, occur in the lipides of the human tubercle bacillus.

Palmitic Acid, $CH_3(CH_2)_{14}.COOH$, m.p. $62^\circ C$., occurs in many plant and animal fats and waxes, especially palm oil, Japan wax, and myrtle wax. It is present in spermaceti as cetyl palmitate and in beeswax as myricyl palmitate. The acid is insoluble in water, slightly soluble in cold alcohol, and easily soluble in hot or boiling alcohol.

Stearic acid, $\text{CH}_3(\text{CH}_2)_{16}\cdot\text{COOH}$, m.p. 69.3°C ., is very widely distributed, especially in the body fat of higher animals as tallow, lard, and suet. It is prepared in quantity by the catalytic hydrogenation or "hardening" of the corresponding unsaturated acids, oleic, linoleic, linolenic, and clupadonic, all of which have the same number of carbon atoms. Stearic acid is a white solid, with a faint but characteristic smell. It is insoluble in water, but easily soluble in boiling alcohol.

(2) **The Oleic Series**, $\text{C}_n\text{H}_{2n-1}\cdot\text{COOH}$.—Unsaturated acids having one double bond, $-\text{CH}:\text{CH}-$. Fifteen members of the series occur naturally.

Oleic acid, $\text{C}_{17}\text{H}_{33}\cdot\text{COOH}$, m.p. 14°C ., the most widely distributed of all the fatty acids. The free acid is a colourless liquid that turns yellow on exposure to air and light. It is a typical example of an *unsaturated* compound, and its presence in glycerides is shown by its property of reducing osmium tetroxide ("osmic acid"), OsO_4 , with the formation of a black deposit, easily recognised in histological preparations.

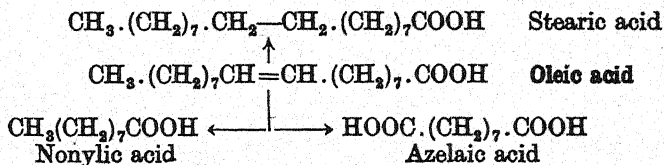
The Structure of Oleic Acid.—(i.) Combustion shows that the empirical formula is $\text{C}_9\text{H}_{17}\text{O}$.

(ii.) Molecular weight determination gives the formula, $\text{C}_{18}\text{H}_{34}\text{O}_2$.

(iii.) Titration shows that a single carboxyl group is present, $\text{C}_{17}\text{H}_{33}\cdot\text{COOH}$.

(iv.) On reduction, oleic acid takes up two atoms of hydrogen, and is converted into the corresponding saturated acid, *stearic acid*, $\text{C}_{17}\text{H}_{35}\cdot\text{COOH}$. This shows that there must be one double bond, or $-\text{CH}=\text{CH}-$ link, in the oleic acid molecule, and that the acid has a straight chain.

(v.) On oxidation, oleic acid breaks up into nonylic acid and azelaic acid, both of which are C_9 acids, indicating that the double bond in oleic acid is midway in the chain.

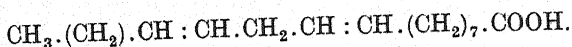


This shows that oleic acid is a $\Delta^{9:10}$ unsaturated acid; the suffix denoting that a double bond occurs between the ninth and tenth carbon atoms, as numbered from the right.

Other examples of the oleic series are: tiglic acid, $\text{C}_8\text{H}_7\cdot\text{COOH}$, from croton oil; erucic acid, $\text{C}_{22}\text{H}_{43}\cdot\text{COOH}$, from mustard oil; and nervonic acid, $\text{C}_{23}\text{H}_{45}\cdot\text{COOH}$, from nervone.

Each member contains one double bond and is converted on reduction into a corresponding member of the stearic series.

(3) **The Linoleic Series**, $C_nH_{2n-3}.COOH$.—The natural acids are all C_{18} compounds with two double-bonds. They do not occur in human storage fat, but are found mostly as phospholipides and as esters of cholesterol. Linoleic acid, the chief member, is a constituent of linseed and other "drying oils," which harden to form a film on exposure to the air. Its formula is



(4) **The Linolenic Series** is represented by linolenic acid, $C_{17}H_{29}.COOH$, which makes up about 20 per cent. of linseed oil, and occurs in all vegetable drying oils. Members of this series have three double-bonds.

(5) **The Arachidonic Series**, having four double-bonds, is represented by arachidonic acid, $C_{19}H_{31}.COOH$, found in lecithin and cephalin.

(6) **The Clupadonic Series**, represented by clupadonic acid, $C_{17}H_{27}.COOH$, in cod-liver oil, and probably all marine oils.

(7) **Hydroxy acids** are represented by the **Cerebronic Series**, including phrenosinic or cerebronic acid from phrenosin of brain tissue; and the **Ricinoleic Series**, including ricinoleic acid, the glyceride of which is the chief constituent of castor oil.

(8) **The Cyclic acids** include the two members, chaulmoogric acid from chaulmoogra oil and hydnocarpic acid from hydnocarpus and chaulmoogra. They are used in the treatment of leprosy.

Aliphatic Alcohols.—Natural fats and oils are esters of the trihydroxy alcohol glycerol; the other aliphatic alcohols occur either free as solutes in oils and fats or combined as *waxes*. Five groups of these lipidic alcohols may be recognised, four of which belong to the straight-chain type of compounds.

(1) Alcohols of the *Ethyl Alcohol Series*, $C_nH_{2n+1}.OH$; these contain one hydroxyl group. Being higher members of the series, they are all odourless and tasteless solids, insoluble in water but soluble in fat solvents. They are characteristic constituents of the waxes. The chief examples are: cetyl alcohol, $C_{16}H_{33}.OH$, from spermaceti; carnaubyl alcohol, $C_{24}H_{49}.OH$, from wool wax; melissyl, or myricyl alcohol, $C_{30}H_{61}.OH$, from beeswax.

(2) Dihydroxy alcohols of the *Glycollic Series*, represented by coryphyl alcohol, $C_{24}H_{48}(OH)_2$, from carnauba wax.

(3) Trihydroxy alcohol of the *Glycerol Series*. Only one member is known, glycerol, the basic constituent of all true fats.

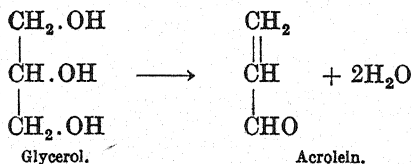
(4) Compound alcohols, α -glyceryl ethers.

(5) Sterols.

Glycerol, or glycerin, $\text{CH}_2\text{OH} \cdot \text{CH}(\text{OH}) \cdot \text{CH}_2\text{OH}$, is quantitatively the most important of the lipid alcohols, and is obtained during the saponification of fats and oils. It is a colourless, odourless, viscid liquid, with a sweet, pungent taste. It is a good solvent, and is very hygroscopic, absorbing about 50 per cent. of its weight of water from the atmosphere. For this reason it is used in cosmetics and "vanishing creams" designed to keep the skin moist and sticky.

Free glycerol is not a lipid. It is insoluble in most of the fat solvents, and is miscible in all proportions with water and with alcohol.

Reactions of Glycerol.—(1) *Dehydration*.—On being heated rapidly along with an anhydrous salt, such as NaHSO_4 , glycerol loses two molecules of water, and is dehydrated to form the unsaturated aldehyde acrolein, which imparts the characteristic acrid smell to burning fat.



(2) *Esterification*.—Glycerophosphoric acid, $\text{CH}_2\text{OH} \cdot \text{CH}(\text{OH}) \cdot \text{CH}_2\text{O} \cdot \text{PO} : (\text{OH})_2$, is prepared by heating glycerol in 60 per cent. phosphoric acid at 100°C . It is a characteristic constituent of the *phosphatides*, such as lecithin.

(3) *Colour Test*.—An aqueous solution of glycerol gives a blue colour on addition of a few drops of 5 per cent. potassium chromate and excess of nitric acid, showing the presence of the primary and secondary alcohol groups.

Nitroglycerin, glyceryl trinitrate, $\text{CH}_2(\text{O} \cdot \text{NO}_2) \cdot \text{CH}(\text{O} \cdot \text{NO}_2) \cdot \text{CH}_2(\text{O} \cdot \text{NO}_2)$, is prepared by adding glycerol to a cooled mixture of nitric and sulphuric acids. It is an ingredient in many high explosives, and is also used therapeutically as a general vaso-dilator.

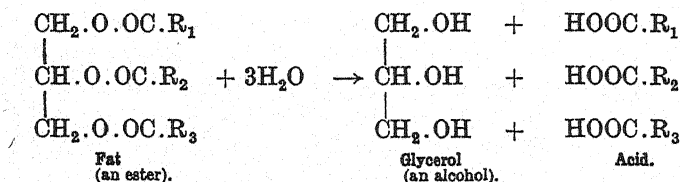
Compound alcohols or alcohol ethers occur in fish-liver oils and bone marrow. For example, *batyl alcohol*,

$\text{C}_{21}\text{H}_{42}\text{O}(\text{OH})_2$, a monoglycerol ether of octadecyl alcohol.

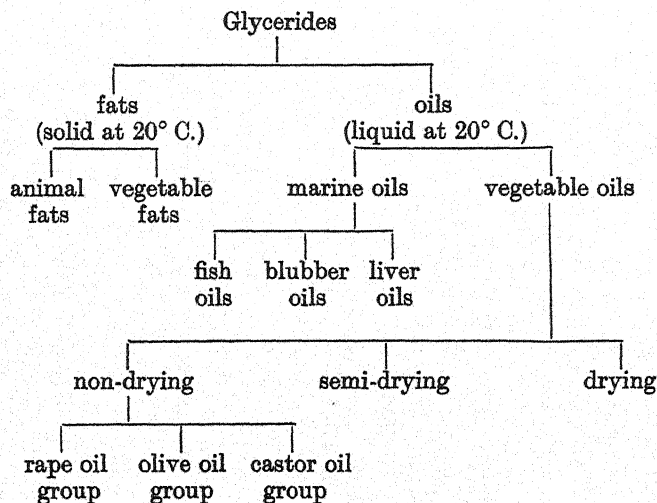
Sterols, or Alcohols of the Cholane Series.—These differ from the other lipid alcohols in the possession of a complex cyclic structure. They are represented by the zoosterols, including *cholesterol*, $\text{C}_{27}\text{H}_{45} \cdot \text{OH}$, found in animals, and the phytosterols and zymosterols, found in plants.

OLEO-LIPIDES AND FATS

These are *glycerides*, that is to say, esters of the trihydroxy alcohol, glycerol, and three molecules of aliphatic acid. On hydrolysis all are resolved into their constituents in accordance with the type equation :



The glycerol may be united to three molecules of the same acid, or three molecules of different acids, the latter constituting a mixed glyceride. Hydrolysis may be accomplished by enzymes, such as the lipases of the alimentary tract, by alkalies, or by superheated steam. Alkaline hydrolysis of a fat is termed **saponification**, because the alkali combines with the liberated aliphatic acid to form a soap.

Classification of Oils and Fats.

The so-called "essential" or volatile oils are not lipides, since they are not esters of aliphatic acids, but are derivatives of cyclic hydrocarbons. They are volatile in steam, and are not saponified by alkalies.

The chief *animal fats* employed industrially are lard, tallow, and butter.

Lard, obtained from the pig, is the mixed glyceride of stearic acid (40 per cent.), oleic acid (50 per cent.), linoleic acid (10 per cent.).

Tallow, or beef and mutton fat, contains mixed glycerides of stearic acid (33–50 per cent.), palmitic acid (10–20 per cent.), and oleic acid (50–60 per cent.).

Butter, or milk fat, occurs to the extent of 3–5.5 per cent. in mammalian milk, and is a complex mixture of the glycerides of oleic, lauric, myristic, palmitic, stearic, and arachidic acids. It is characterised by the presence of butyric acid, which makes up 4–6 per cent. of the total fatty acid present.

Human fat resembles tallow in many respects; its fatty acid content being largely determined by the fatty acids in the diet. At body temperature human fat is almost liquid, owing to its high percentage of glycerides of oleic acid.

Vegetable Fats.—The principal members of a large group are:—

Cacao butter (cocoa butter), from the beans of the cocoa tree (*Theobroma cacao*), is made of glycerides of stearic and oleic acids. It is used in pharmacy and in confectionery.

Cocoanut oil, from the fruit of the cocoanut tree (*Cocos nucifera*), is used largely in the preparation of edible fats, margarines, and “nut butter.”

Vegetable oils are divided into *drying oils*, which harden on exposure to light and air, and *non-drying oils*, which do not. Various intermediate forms are grouped as *semi-drying oils*. The property of “drying” depends on the presence of highly unsaturated acids in the glyceride.

Important non-drying oils are *rape seed oil* or *colza oil*, from the mustard; *almond oil*, *olive oil*, and *castor oil*, from seeds of *Ricinus communis*. Many of these are used as lubricants.

The commonest drying oils are *linseed oil*, from flax seed (*Linum usitatissimum*), *tung oil*, *hempseed oil*, *soya bean oil*, and *walnut oil*.

Marine oils include the *fish oils*, found almost uniformly distributed in the tissues of most fish; *liver oils*, chiefly from cod, shark, ling, halibut and skate, are of great therapeutic value as sources of the vitamins A and D; *blubber oils*, from the oleaginous tissues of the seal and the whale.

ANALYTICAL REACTIONS OF SIMPLE LIPIDES

Alkaline Hydrolysis (Saponification).—Shake up 5 ml. of olive oil or melted fat with 5 ml. of 20 per cent. sodium hydroxide. A white emulsion forms, consisting of a disperse phase of liquid fat in a continuous aqueous phase. Immerse the tube in boiling water. The emulsion is unstable and resolves into a layer of oil on a layer of alkali. Hydrolysis takes place at the interface, and

is aided by shaking the tube occasionally. After at least half an hour, remove and cool the tube. The contents show three layers : a lower one of alkali and glycerol, an upper one of unchanged oil, and an intermediate solid layer of soap, sodium oleate, which has been "salted-out" of the lower layer by the excess of alkali. Carefully pour off the liquid layers, and test for glycerol by means of the nitro-chromic test (p. 122). Rinse the small cake of soap with cold water to remove excess of alkali. Then add about 10 ml. of water, and boil till the soap dissolves.

Detection of Soap.—(a) Acidify about 3 ml. of solution with a few drops of HCl. The soap is decomposed and the liberated fatty acid separates out as a white precipitate.

(b) Add a few drops of 5 per cent. CaCl_2 to 3 ml. of soap solution. A white precipitate of calcium soap separates out. This reaction takes place in "hard" water when soap is added, and renders washing uneconomical.

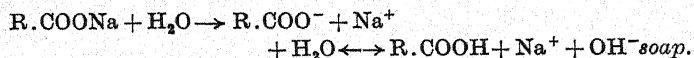
(c) Add about 1 ml. of saturated NaCl to 3 ml. of soap solution. The soap is displaced, or "salted-out," and rises to the top of the liquid. This process is employed industrially in the purification of soap.

Detection of Glycerol.—Add a few drops of 5 per cent. potassium chromate and an excess of concentrated nitric acid to the mixture of oil and alkali after separation of the soap. A blue colour develops in the aqueous layer owing to the presence of glycerol liberated from the hydrolysed fat.

SOAPS

A soap is the metallic salt of a higher aliphatic acid, and is formed whenever fats are hydrolysed in an alkaline medium. Potassium, sodium, and ammonium soaps are dissolved readily by water to form colloidal solutions, and are used in washing. Calcium and magnesium soaps are insoluble, and represent a form in which these metals are excreted by the intestine.

Soaps form alkaline solutions, owing to removal of H^+ by the aliphatic anions to produce the feebly ionised aliphatic acid :



WAXES

Waxes are formed by union of a higher alcohol and an aliphatic acid, and are non-glycerides. All are insoluble in water but soluble in fat solvents. They are much more resistant to hydrolysis than true fats, and are not attacked by the lipoclastic enzymes, hence, waxes are not utilisable in animal nutrition. When hydrolysed they show their ester structure by giving rise to an aliphatic acid, and an alcohol.

The chief waxes are wool wax, beeswax, spermaceti, chinese wax, and carnauba wax.

Wool wax, or *lanolin*, comes from the fleece of sheep. It is a mixture of esters and free alcohols, including the sterol cholesterol, also lanosterol and agnosterol, which are derivatives of a 5-ring hydrocarbon, *picene*.

Lanolin may absorb up to 80 per cent. of its weight of water, and is an important agent for the dermal administration of drugs. It is absorbed by the skin, and for this reason is hopefully described as a "skin food" by makers of cosmetics.

Beeswax, secreted by the honey-bee during digestion, is used for building the comb. It is chiefly myricyl palmitate, and is largely employed in the manufacture of polishes.

Spermaceti, obtained as a solid from the head oil of the sperm and other whales, is chiefly cetyl palmitate. Its principal industrial use is the manufacture of candles.

COMPLEX LIPIDES, OR LIPINES

Complex lipides resemble the fats physically, and yield aliphatic acids on hydrolysis. They differ chemically from the simple lipides in containing phosphoric acid or galactose in the molecule, usually associated with a basic nitrogen compound. The presence of nitrogen is indicated in the alternative name *Lipine*.

(1) **Phospholipides** are recognised and separated from other lipides by two chief properties :—

(i.) Insolubility in cold acetone. They may be precipitated from ethereal extracts of mixed lipides by addition of acetone.

(ii.) On hydrolysis they liberate phosphoric acid, the proportion being one molecule of acid for each molecule of lipine.

Four sub-groups of phospholipides are known :—

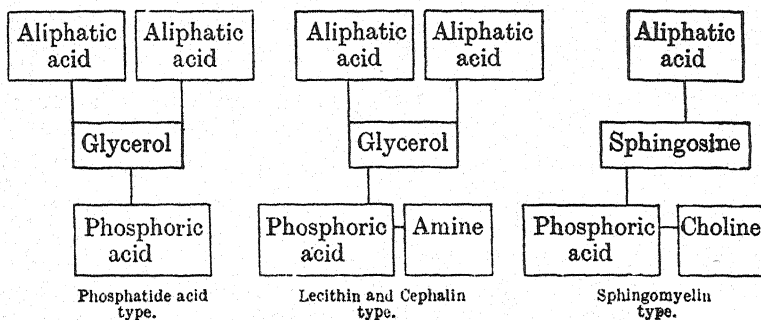
(a) **Lecithins**, glycerol esters of aliphatic acids and choline phosphate. They are universal in plants and animals, especially in embryonic tissue, nervous tissue, bone marrow, and egg-yolk, which may contain about 9 per cent.

(b) **Cephalins**, glycerol esters of aliphatic acids and colamine or serine phosphate. They are abundant in brain and nervous tissue, and in egg-yolk. Cephalin, unlike any other phospholipide, has a powerful coagulative action on blood, and is believed by Howell to be identical with thrombokinasase

(c) **Sphingomyelins**, amides of an aliphatic acid with sphingosine choline phosphate, are abundant in brain tissue, and closely associated with the glycolipides, forming the mixture "protagon."

- (d) **Phosphatidic acids**, or phosphotidates, esters of aliphatic acids and glycerophosphoric acid. They are found in vegetable lipoids from seeds, fruit, and leaves.
- (e) **Bacterial phosphatides** separated from the waxy lipides of the *Tubercle Bacillus*. On hydrolysis they yield: palmitic acid, oleic acid, and *phthioic acid*, a branched-chain aliphatic acid that stimulates tubercle-formation in the tissues. These compounds differ from all the other phosphatide types.

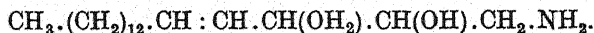
Structure of the Phospholipides.



Phospholipide Bases.—(1) *Colamine*, or ethanolamine, $\text{HO} \cdot \text{C}_2\text{H}_4 \cdot \text{NH}_2$ occurs in cephalins, possibly as a precursor, *serine*.

(2) *Choline*, $\text{HO} \cdot \text{C}_2\text{H}_4 \cdot \text{N}(\text{CH}_3)_3\text{OH}$, is found in lecithins. It and its derivative, *acetyl choline*, are of great physiological interest.

(3) *Sphingosine* is an 18-carbon amine containing two hydroxyl groups,

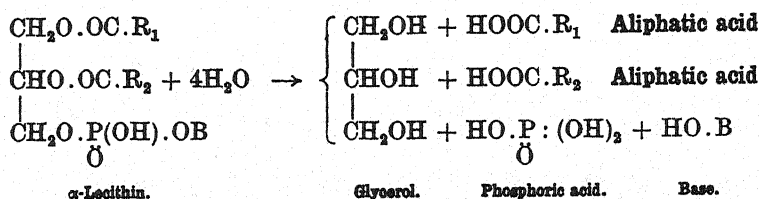


Lecithins are phospholipides containing choline as the only nitrogenous base. Each lecithin contains one molecule of glycerol, two of aliphatic acid, one of phosphoric acid and one of choline. These radicles are assembled in one of two ways. In the α -lecithins the phosphoric acid is joined to the terminal or α -carbon of the glycerol; in the β -lecithins, it is joined to the central or β -carbon atom. The aliphatic radicles differ in lecithins from different sources. Thus, egg-yolk lecithin contains stearic and oleic acid, liver lecithins contain palmitic or stearic acid along with oleic or arachidonic acid.

Lecithins are separated from the other phospholipides by precipitation from alcoholic solution on addition of cadmium chloride. When pure, they are white, waxy solids, very hygroscopic and liable to oxidation.

They form emulsions spontaneously when mixed with water.

Hydrolysis of Lecithin.—Lecithin is attacked by lipase, which liberates the aliphatic acids, and by phosphatase, which liberates the phosphoric acid. Strong alkalis bring about complete hydrolysis in accordance with the equation :—



R_1 and R_2 are the acid radicles, *palmitic*, *stearic*, *oleic*, etc., as may be. B is the radicle of *choline*, $-\text{CH}_2.\text{CH}_2.\text{N}^+(\text{CH}_3)_3$.

Cobra venom contains an esterase capable of liberating the unsaturated aliphatic acid from lecithin, and leaving a residual *lysolecithin*. Lysolecithins are powerful hæmolysins, and contribute to the fatal effect of injection of snake venom. They are antagonised by cholesterol, with which they form non-hæmolytic compounds.

Functions of Lecithin.—Lecithin is concentrated chiefly in egg-yolk, medullary sheaths of nerves and liver tissue. A 50-gm. egg contains about 1 gm. of lecithin in its 15-gm. yolk. The lipine forms a storage compound which is drawn on as the embryo develops.

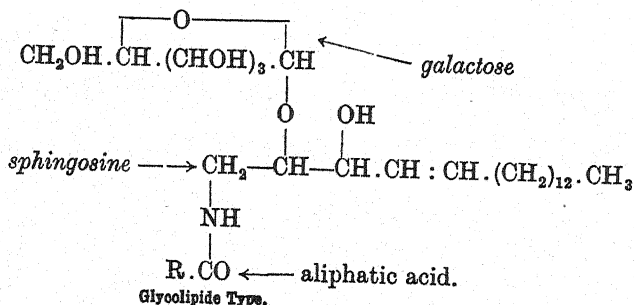
In nervous tissue it provides a reserve supply of choline, which in turn gives rise to the neurocrine, acetyl choline, necessary for the transmission of the nerve impulse.

Sphingomyelin forms about 1-1.5 per cent of fresh brain and other nerve tissue, and also occurs chiefly in lung, where it represents about one-third of the phospholipides. There is no relation between total phospholipide and sphingomyelin content of the various tissues, which suggests that sphingomyelin has a function different from the other phospholipides.

(2) **Glycolipides** are of a glycoside structure, and on hydrolysis liberate: (a) galactose, a reducing sugar; (b) sphingosine; and (c) an aliphatic acid. They occur chiefly in brain tissue, making up about 2-4 per cent. of fresh material, and are essential constituents of the medullary sheaths of nerves. Glycolipides, unlike the other lipides, are sparingly soluble in ether, and form part of the residue left after the ethereal extraction of brain substance. They are removed by means of warm alcohol, from which by fractional crystallisation they can be separated into: *phrenosin*, *kerasin*, *nervone*, and *hydroxynervone*.

Phrenosin, $C_{48}H_{91}NO_8$, is a white, micro-granular powder. On hydrolysis it yields: (a) galactose; (b) sphingosine; and (c) phrenosinic acid.

Kerasin, *nervone*, and *hydroxynervone* resemble phrenosin but for the fact that each contains a different aliphatic acid united to the sphingosine galactoside. This is explained by means of the type formula:—



The nature of R, the aliphatic acid residue, depends on the glycolipide, as follows:—

Kerasin yields lignoceric acid, $C_{23}H_{47} \cdot \text{COOH}$.

Phrenosin yields phrenosinic, or cerebronic, acid.

Nervone yields nervonic acid, $C_{23}H_{45} \cdot \text{COOH}$.

Hydroxynervone yields α -hydroxynervonic acid.

Glycolipides occur principally in the myelin sheaths of nerves. They were first isolated by Thudichum (1901).

Biological Significance of the Lipides.—A. Simple lipides.

- (1) *Food Material*.—Fat in its various forms is one of the three great sources of carbon in the dietary. Containing less oxygen than the carbohydrates or the proteins, it is a highly concentrated form of fuel, 1 gm. liberating on combustion about 9.3 kilocalories of energy.
- (2) *Food Reserve*.—On account of its insolubility in aqueous solutions, fat is stored readily in the organism, and is available to meet nutritional requirements. The adult man in health has a fat-content of about 120 gm. per kg. body weight; more than half of this is reserve or storage fat.
- (3) *Heat Insulation*.—The subcutaneous lipides of the organism retard loss of heat from the surface of the body. They are specially concentrated in aquatic mammals, such as the whale, and in animals living in cold climates.
- (4) *Solvents*.—Dietary fat and storage fat carry in solution many important solutes, notably the fat-soluble vitamins A, D, and E.

B. Complex Lipides

- (5) *Structural Constituents*.—While storage fat is readily leached out of the dried tissues by extraction with ether or chloroform, some lipide material remains behind, and can only be removed completely by boiling alcohol. This is chiefly phosphatides, glycolipides, and steroids, and is believed to form part of the cell framework.
- (6) *Fat Transport*.—There is evidence that the phospholipide of the blood stream aids in the absorption and transportation of the aliphatic acids.
- (7) *Fat Metabolism*.—Fats are converted into phospholipides before undergoing desaturation and oxidative degradation.
- (8) *Storage Substrates*.—Choline and other reagents are held in an insoluble form in lipines until released by the appropriate enzymes.

CHARACTERISATION OF THE LIPIDES

The natural lipides, on account of their intersolubility, occur in mixtures that, for the purpose of analysis, are submitted to a routine series of estimations.

(1) *Acid Value*.—The amount of potassium hydroxide, in mg., required to neutralise the free fatty acids present in 1 gm. of the material.

This value measures the rancidity and degree of hydrolysis of the fat.

(2) *Saponification Value*.—The amount of potassium hydroxide, in mg., required to saponify completely 1 gm. of the fat. This measures the total fatty acids present in ester and free form, and varies inversely with the molecular weight of the fatty acids.

(3) *Iodine Value*.—The amount of iodine, in gm., that is decolourised by combining with 1 gm. of fat. This measures the degree of unsaturation of the acids present in the fat. The process is catalysed by using the iodine as a monobromide or monochloride.

(4) *Volatile Acid Value*, or Reichert-Meissl Number.—The amount of N/10 K. OH required to neutralise the volatile fatty acids obtained when 5 gm. of hydrolysed fat is distilled by a current of steam. This measures the total fatty acids of low molecular weight up to C₁₂.

(5) *Thiocyanogen Value* (Kaufmann, 1925).—The weight of thiocyanogen, (SCN)₂, absorbed by 1 gm. of fat. This is a better

index of the degree of unsaturation than the iodine method, which is often inadequate for fatty acids with more than one double-bond.

Analytical Constants for Common Fats (Plimmer, 1938)

Fat.	Saponification Value.	Iodine Value.	Reichert Value.
Lard	195	46-70	0.6
Olive oil	185-196	79-88	0.6
Linseed oil	192-195	173-201	0.0
Butter	220-233	26-50	26-33

Mixed glycerides, such as make up animal fats, are described briefly in terms of their component acids. Thus, *tristearin* is the glyceride containing three stearyl residues, while β -*palmito- α -distearin* has a palmityl unit in the central, or β -position, and a stearyl unit at each of the ends of the glyceryl residue.

Fats are also characterised by their *refractive index* and by their *specific gravity*, which for a solid fat is about 0.85 and for an oleo-lipide about 0.91 to 0.94. After liberation by hydrolysis and acidification, the fatty acids are isolated by repeated fractionation of their lithium or lead salts, or by low-temperature crystallisation. Thus, when an acetone solution of mixed acids from a natural fat is cooled to about -30°C ., the saturated acids separate, while further cooling from -40° to -80°C . removes unsaturated acids (J. B. Brown, 1937).

Fats as a class have lower melting-points than those of their constituent acids, and re-solidify, at temperatures below their m.p. Human fat melts about 17°C ., and, hence, is in the liquid state at body-temperature. When a cold-blooded animal, such as a frog, is kept in a rising-temperature environment, its metabolic rate changes abruptly at points corresponding to the melting-points of its tissue fat (O'Connor, 1943).

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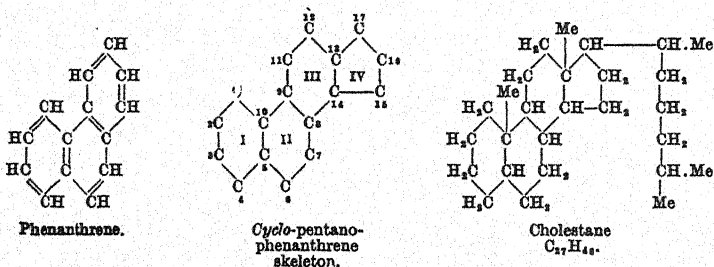
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CHAPTER 11

STEROIDS

THE lipid fraction obtained when tissues are extracted with fat-solvents usually contains, in addition to simple and complex fats, a varying amount of "lipoid" material that is not saponified by alkalis, and which consists partly of *sterols*, or solid alcohols derived from a saturated cyclic hydrocarbon, *cholestane*, $C_{27}H_{48}$. The first sterol to be recognised was obtained from gall stones, and was called "cholesterine" (Gk. *solid bile*), a name later changed to **cholesterol**, when an alcohol group was shown to be present in the compound. The generic term **sterol** was subsequently applied to substances resembling cholesterol. Eventually it was discovered that other important biological compounds, such as bile acids, hormones and vitamins, were closely related to cholesterol in that they contained the cyclic nucleus present in cholestane, but did not possess the long side-chain present in all sterols. These compounds are now termed **steroids**. Finally, the parent nucleus of all the sterols and steroids was identified as *cyclo-pentanophenanthrene*, a saturated hydrocarbon containing three 6-membered rings in phenanthrene configuration, and a 5-membered associated ring.

Definition.—Sterols and steroids are derivatives of a tetra-cyclic hydrocarbon, *cyclo-pentanophenanthrene*, $C_{17}H_{28}$, which in the sterols is methylated and carries a side-chain, thus constituting the *cholestane* system.



Classification.—Steroids differ so profoundly in their physiological activity that a chemical classification tends to obscure their practical

significance, and they are provisionally classified according to distribution and function.

Sterols.	Steroids.	
(1) Zoosterols	(4) Bile acids	(7) Toad poisons
(2) Phytosterols	(5) Saponins	(8) Provitamins D
(3) Zymosterols	(6) Cardio-toxic glucosides	(9) Sex hormones
		(10) Carcinogens

STEROLS

Sterols occur universally in animals and plants, often dissolved in the storage lipides, or present in the form of esters in tissue fluids and organs. They are colourless, waxy solids, insoluble in water, and soluble in fat solvents. Chemically and biologically they are quite distinct from the simple and complex lipides, and their association with these compounds is due to their physical similarity. They are sub-divided into (i.) *zoosterols*, found in animals; (ii.) *phytosterols*, found in higher plants; and (iii.) *zymosterols*, or *mycosterols*, found in lower plants, such as fungi and moulds. They are all optically active compounds.

Natural Sterols

Sterol.	Formula.	M.p.	$[\alpha]_D$	Chief Sources.
<i>Zoosterols :</i>				
Cholesterol . . .	$C_{27}H_{45}.OH$	148°	-37°	All animal tissues
Dihydrocholesterol . .	$C_{27}H_{47}.OH$	142°	+28°	" "
Coprosterol . . .	$C_{27}H_{47}.OH$	100°	+24°	Fæcal lipoids
Spongosterol . . .	$C_{27}H_{47}.OH$	124°	-19.6°	Zoophytes
Ostreasterol . . .	$C_{29}H_{47}.OH$	143°	-44°	Molluscs
7-dehydrocholesterol	$C_{27}H_{43}.OH$			Liver Oils
<i>Phytosterols :</i>				
Sitosterol . . .	$C_{29}H_{49}.OH$	138°	-24°	Higher plants, wheat germ
Stigmasterol . . .	$C_{29}H_{47}.OH$	170°	-45°	Soya bean, Calabar bean.
<i>Zymosterols :</i>				
Ergosterol . . .	$C_{28}H_{43}.OH$	161°	-180°	Yeast, ergot

Sitosterol occurs as a mixture of α -, β - and γ -isomers.

Cholesterol.—This sterol was isolated from biliary calculi in 1775, and has attracted continuous attention for over two hundred years; partly on account of its surgical importance as a constituent of gall stones, partly because of its unusual and complex chemical structure, and partly because it is a universally distributed zoosterol of obscure origin and significance.

Its ubiquity in the animal kingdom was demonstrated by Dorée,

in 1909. Cholesterol occurs normally free or combined (usually as an oleate or a palmitate) in tissues and secretions, notably brain, bile, blood and adipose deposits; it is found pathologically in biliary calculi, sebaceous cysts and atheromatous blood vessel walls.

Representative cholesterol values, expressed as percentage, are: human brain, fresh, 2.2; human brain, dried, 10.9; suprarenal gland, fresh, 5-7; sciatic nerve, dried, 5.6; spleen, kidney and lung, fresh, 0.3-0.5; animal fats, 0.1-0.35; human bile, 0.06-0.16; human milk, 0.03; blood plasma, 0.07-0.08; egg-yolk, fresh, 0.49; liver oils, 0.5-1.5.

In acute nephritis accompanied by cedema, the cholesterol content of the plasma increases in proportion to the cedema, and may reach a value of 390 mg. per 100 ml. Where there is no cedema, the cholesterol value may be unchanged. In chronic parenchymatous nephritis, the cholesterol level may rise as high as 730 mg. per 100 ml. plasma.

Cholesterol has not yet been found in any plant tissues, and, as Dorée has pointed out, its presence in a vegetable oil is evidence of adulteration with fat of animal origin.

Cholesterol is obtained readily by extracting powdered biliary calculi with boiling alcohol. On cooling, the sterol crystallises out in characteristic flat plates with one corner notched.

Origin of Cholesterol in Animals.—Preformed cholesterol in the mixed diet is believed to be sufficient to meet the sterol requirements of the adult animal, any excess being excreted by the intestine, either unchanged or reduced by bacterial action. Among herbivora, cholesterol does not occur in the diet, and must arise either by direct synthesis or by conversion of the vegetable phytosterols. Channon has shown that animals on a cholesterol-free diet are able to synthesise the sterol. Metabolic cholesterol is excreted in the bile, and enters the intestine, where it is changed by bacterial hydrogenation into the dihydro-derivative, coprostanol, formerly termed *coprosterol*, in which form it leaves the body.

Sitosterol and Stigmasterol are characteristic of higher plants, and are concentrated in tissues rich in lipides, especially the germ of the seeds. These sterols are restricted to the plant kingdom, and although they necessarily occur in the animal diet, there is no evidence either of their utilisation or conversion to zoosterols by higher animals, although it is possible that certain molluscs unable to manufacture cholesterol may obtain their characteristic *ostreasterol* from the isomeric phytosterols that occur in the algæ on which they feed.

Ergosterol, Provitamin D₂.—This sterol was isolated from the fungus *Ergot* by Tanret, but attracted little attention until 1927,

when it was shown to be converted into vitamin D₂ by solar or ultraviolet irradiation. It is found in many moulds, yeasts, higher plants, and in the liver oils and nervous tissues of animals. It crystallises readily, and can be separated from cholesterol and other sterols owing to its non-precipitability by digitonin, and its lower solubility in alcohol. Unlike cholesterol, it has three well-marked absorption bands in the ultra-violet region of the spectrum.

ANALYTICAL REACTIONS OF THE STEROLS

Cholesterol.—Cholesterol forms a characteristic precipitate with the alkaloid digitonin, by means of which it may be isolated and estimated.

(1) *Acetic anhydride test* (Liebermann and Burchard)—About 5 drops of acetic anhydride are added to 1 ml. of a solution of cholesterol in chloroform. After mixing, concentrated sulphuric acid is added drop by drop until a violet colour appears. The violet soon changes into a stable emerald green, which has been employed for the colorimetric estimation of the sterol.

(2) *Trichloroacetic acid test* (Rosenheim, 1929)—Cholesterol and other natural sterols develop a red colour on being warmed with an excess of 90 per cent. trichloroacetic acid.

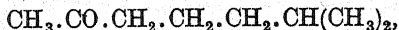
Saturated sterols, such as coprosterol, do not give these tests.

Ergosterol.—(1) *Chloral Hydrate Test* (Rosenheim, 1929).—When 1 mg. or less of ergosterol is added to about 1 gm. of chloral hydrate melted in a water-bath, a carmine colour is produced which shows an absorption band at 500 mμ. This red colour soon changes into green, and then into a deep blue. A few natural sterols develop a red colour on warming with chloral hydrate, but the change to blue is seen only with ergosterol.

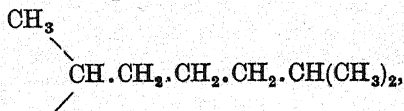
(2) *Bromine Test* (Tortelli-Jaffé).—A crystal of ergosterol is dissolved in 5 ml. of glacial acetic acid, and 1 ml. of a 2 per cent. solution of bromine in chloroform is added. A green ring appears at the surface of contact between the liquids.

Structure of the Sterols

(a) *The Side-chain.*—Cholesterol on oxidation yields an aliphatic ketone and a benzenoid residue. The former was identified by Windaus (1912) as methyl *iso*-hexyl ketone,



which indicates that the parent side-chain is



oxidation having taken place at the point of attachment of the $-\text{CH}$ group to the stable nucleus. By a similar procedure, the side-chains of the other sterols, and the steroid *cholic acid*, were separated and identified, and the following semi-structural formulæ were deduced :—

Cholesterol $R.\text{CH}(\text{CH}_3).\text{CH}_2.\text{CH}_2.\text{CH}_2.\text{CH}(\text{CH}_3)_2.$

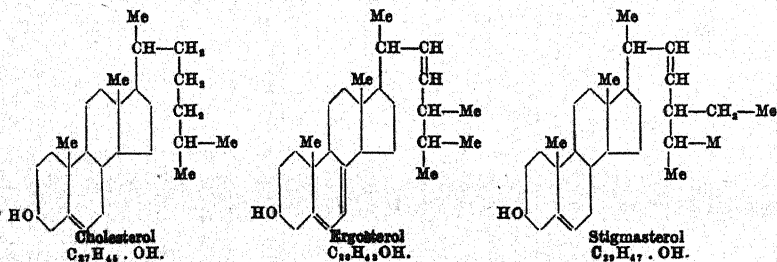
Stigmasterol $R.\text{CH}(\text{CH}_3).\text{CH}=\text{CH}.\text{CH}(\text{C}_2\text{H}_5).\text{CH}(\text{CH}_3)_2.$

Ergosterol $R.\text{CH}(\text{CH}_3).\text{CH}=\text{CH}.\text{CH}(\text{CH}_3).\text{CH}(\text{CH}_3)_2.$

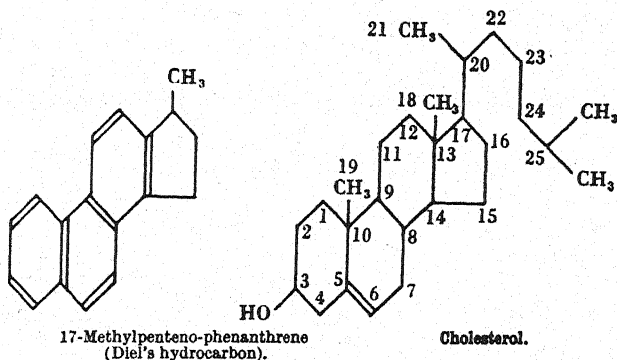
Cholic acid $R.\text{CH}(\text{CH}_3).\text{CH}_2.\text{CH}_2.\text{COOH}.$

(b) **The Nucleus.**—Subtracting the value of the side-chain, C_8H_{17} , from the formula for cholesterol, the value $\text{C}_{19}\text{H}_{28}.\text{OH}$ is obtained for the nucleus. The stability of the sterol, and the ratio of C to H in the nucleus suggest the presence of four hydrogenated rings fused together. One of these rings carries the hydroxyl group, and on oxidation is broken at this point, giving rise to a monocarboxy acid. By chemical methods this acid can be degraded until it has lost two $-\text{CH}_2$ groups, showing that it has the side-chain, $-\text{CH}_2.\text{CH}_2.\text{COOH}$, attached to a second ring (II). Since this side-chain was formed by the opening up of another ring at its weakest point, the position of the hydroxyl group in the original ring (I) can be located. *Lithocholic acid* contains a similarly placed hydroxyl group, and its nucleus can be oxidised and degraded in the same way. Cholic acid, however, contains three hydroxyl groups in its nucleus, and the oxidation derivatives indicate that each of them is attached to a different ring, which suggests the presence of a phenanthrene system of three benzenoid rings. Meanwhile, the preparation of cholestane and other reduction derivatives from various steroids supported the belief that the nucleus of all these compounds was similar in pattern, although the existence of various isomeric forms aggravated the difficulties of the research.

It was not found possible to assign a benzenoid structure to the remaining ring (IV). In 1932, Rosenheim and King suggested that rings I, II and III occurred as a phenanthrene system, and, subsequently, Wieland completed the formula by showing that ring IV was derived from cyclo-pentane.



When cholesterol is dehydrogenated by Se at $320^{\circ}\text{C}.$, it yields a cyclic compound, $\text{C}_{18}\text{H}_{16}$, known as Diel's hydrocarbon, which, in 1934, was shown by Harper, Kon and Ruzicka to be identical with synthetically-made 17-methylpenteno-phenanthrene' thus confirming the 4-ring structure ascribed to the steroid nucleus.



Formulation of the Steroids.—The method adopted for numbering the carbon atoms in the nucleus and side-chains is shown. In cholesterol, the nucleus carries a methyl group at 10 and 13, and a hydroxyl at 3. There is a double-bond between 5 and 6 in ring II; apart from this, both the nucleus and side-chain are fully saturated. Hence, cholesterol is the 3-hydroxy-10 : 13-dimethyl- $\Delta^{5:6}$ -derivative of the parent cyclic hydrocarbon.

The natural sterols and steroids differ in the attached side-chains, and in the number and position of the unsaturated bonds, but, as far as is known, all conform to a general type :

(1) In ring I, carbon 3 is always attached to oxygen, either as $-\text{OH}$ or as $=\text{O}$, which is denoted by *-ol* or *-one*, respectively, in the name of the compound.

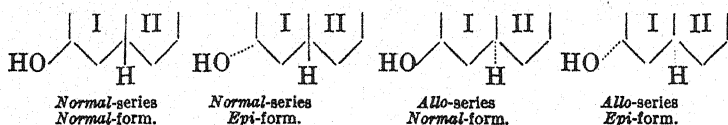
(2) Carbon 13 always carries a methyl group.

(3) Carbon 17, in ring IV, is always joined to a side-chain, as in the sterols, or carries $-\text{OH}$ or $=\text{O}$.

(4) Carbon 10 carries $-\text{CH}_3$ in sterols, bile acids, adrenal cortex hormones, and male sex hormones, but is only occupied by H in the female sex hormones. Rings III and IV are saturated and very stable. Rings I and II are unsaturated in varying degrees; while in vitamins of the D class, ring II has been opened up, thereby destroying the steroid nucleus.

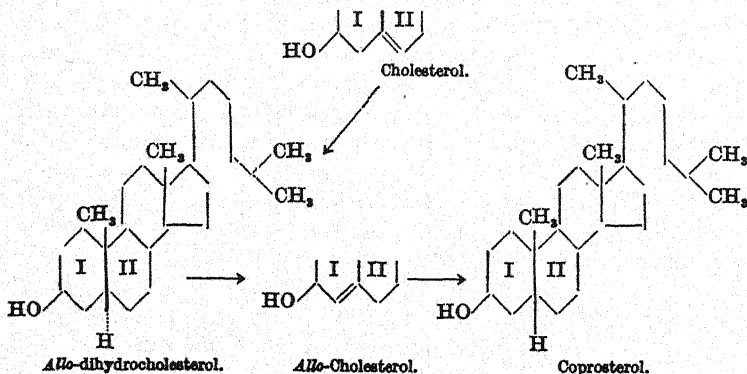
Isomerism of the Steroids.—Apart from the side-chain, the cholestane ring system has, when fully saturated, seven asymmetric carbon atoms (5, 8, 9, 10, 13, 14, 17), which implies the possible

existence of 2^7 , or 148, isomers. In the known natural steroids, however, isomerism is restricted to the configuration at C5, and at C3, whenever this carbon carries an —OH group. Isomers of C5 are described as belonging either to the *normal-* (*cis*) or *allo-* (*trans*) series; while C 3 isomers are distinguished as being either *normal-* or *epi-*forms of the *normal-* or *allo-series*. In formulation, this is shown by an unbroken line of attachment for the —H at 5-or the —OH at 3 in the *normal-series*; while in the *allo-series* or the *epi-*form, attachment is shown by dotted lines, indicating that the direction is different. As this isomerism in natural compounds only concerns rings I and II, it can be shown briefly :—



Cholesterol is taken as being a *normal-form* of the *normal-series*. On reduction, the double-bonds at 5 : 6 are reduced by acceptance of two H atoms, yielding *allo-dihydrocholesterol*, which accompanies cholesterol in animal tissues.

In the intestine, coprosterol, or *normal-dihydrocholesterol*, occurs, and is attributed to the result of hydrogenation by bacteria. Since cholesterol on reduction yields the *allo-isomer*, Schoenheimer (1938) concludes that coprosterol-formation involves the production of *allo-cholesterol*, the $\Delta^{4:5}$ -isomer of cholesterol, as an intermediate.



Ergosterol (provitamin D_2) differs from cholesterol by an additional methyl group and two double bonds, one of which is in the side-chain. Stigmasterol differs from cholesterol only in the side-chain, which has a double bond and an additional ethyl group.

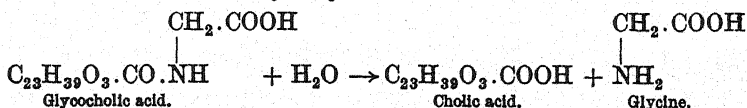
STERIODS

The Bile Acids.—The bitterness of gall was proverbial when Israel dwelt in Egypt, and is a characteristic property of the bile of all animals. It is due to the presence of *glycocholic acid* and *taurocholic acid*, which are manufactured in the liver. On hydrolysis, these give rise to a parent bile acid, **cholic acid**, which is often accompanied by related acids. Methods of estimation are not yet entirely satisfactory, but it is believed that human fistula bile contains 0.5 to 1.0 per cent. of the bile acids, present as esters, or "bile salts," chiefly glycocholate.

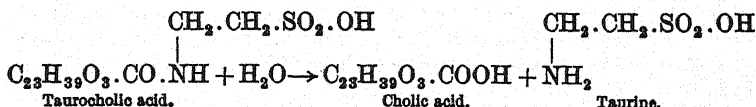
Natural Bile Acids

Acid.	Formula.	Source.
Cholic acid . . .	$C_{26}H_{46}(OH)_3.COOH$	Man, ox, goat, sheep
Desoxycholic acid . . .	$C_{25}H_{42}(OH)_2.COOH$	Man, ox, "goose," hen
Cheno-desoxycholic acid . . .	$C_{25}H_{42}(OH)_2.COOH$	Man, ox
Lithocholic acid . . .	$C_{25}H_{42}(OH).COOH$	Pig
Hyodesoxycholic acid . . .	$C_{25}H_{42}(OH)_2.COOH$	Shark
Scymnol . . .	$C_{26}H_{46}O_3.COOH$	

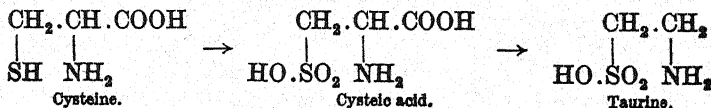
Glycocholic acid is derived from glycine and cholic acid, and is resolved into them on hydrolysis.



Taurocholic acid is a derivative of taurine and cholic acid :



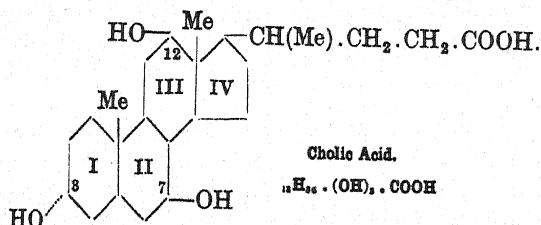
Taurine, amino-ethylsulphonic acid, is derived from the amino acid cysteine, by oxidation to cysteic acid, followed by decarboxylation :



Structure of the Bile Acids

Bile acids are *epi*-hydroxy derivatives of a saturated **cholanic acid**, which has the same nucleus as cholestane, but the side-chain has been shortened to $—CH(CH_3).CH_2.CH_2.COOH$.

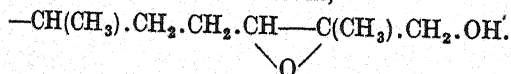
Cholic acid is a trihydroxy cholanic acid in which the hydroxyl groups occupy positions 3, 7 and 12 in the nucleus.



Mild oxidation of cholic acid converts the three —OH groups into $=\text{O}$ groups, forming *dehydrocholic acid*, a powerful cholagogue.

The acids which accompany cholic acid differ in their degree of reduction. Thus, *desoxycholic acid* has OH at 12 replaced by H ; *cheno-desoxycholic acid*, has the OH at 7 replaced by H ; while *lithocholic acid* has the —OH group at 7 and at 12 replaced by H .

Scymnol, $\text{C}_{27}\text{H}_{46}\text{O}_5$, occurs as a sulphuric ester in shark bile, and is of interest in that it represents a compound intermediate in type between cholesterol and cholic acid, and may indicate their biochemical relationship. Scymnol has the same nucleus as cholic acid, but carries a different side-chain,



Preparation of Bile Acids.—Mix a paste made of 50 ml. of ox bile and 10 gm. of animal charcoal, and evaporate to dryness on a water-bath, stirring at intervals. Powder the residue, and boil with 100 ml. of 95 per cent. alcohol for about half an hour. Cool, and filter into a dry dish. Add ether until the mixture begins to form a permanent cloud. Cover, and leave overnight at low temperature. A crystalline mass of bile acids separates out, and may be filtered off, and purified by reprecipitation from alcohol.

The acids are separated by fractional crystallisation from water, in which taurocholic acid is much more soluble than glycocholic acid. Taurocholic acid is very bitter; glycocholic acid has a characteristic bitter-sweet taste. It is toxic, being cytolytic and hæmolytic, an effect seen in the red cell destruction in hæmatogenous jaundice. It slows the heart by stimulating the vagus.

Choleic Acids, which also occur in bile, are water-soluble complexes of desoxycholic acid and aliphatic acids.

Steroid Glucosides

The Neutral Saponins.—Soapwort (*Saponaria*) and many other plants contain a class of glucoside characterised by the property of yielding soap-like froths in aqueous solution, and capable of acting as emulsifiers and detergents, and hæmolyzing blood cells, even in very low concentration.

On hydrolysis, saponins yield glucose and an aglucone (p. 110) termed a *genin*. Thus, the common foxglove (*Digitalis purpurea*) contains a group of saponins, including *digitonin*, which yields *digitogenin*, $C_{27}H_{44}O_5$.

The Cardio-toxic Glucosides.—Saponins are often associated with glucosides having a powerful action on the heart, especially in plants belonging to the order *Apocyanaceae*.

The most familiar of the aglucones obtained from these glucosides belong to the digitalis group, used for over a hundred years in medicine, and the strophanthus group, which has a longer history as a source of arrow poisons. Representative members are *digitoxigenin*, $C_{23}H_{34}O_4$, and *strophanthidin*, $C_{23}H_{32}O_6$, but at least nine have been isolated and identified.

Both the saponin genins and the cardio-toxic aglucones possess the cyclo-pentanophenanthrene nucleus characteristic of the sterols, to which is affixed a side-chain which determines the specific properties of the steroid. Thus, cardio-toxicity is associated with the presence of an unsaturated lactone ring attached to ring IV of the nucleus.

The Toad Poisons.—The "ugly and venomous" toad owes its reputation to the presence of *bufotoxin* in the secretion of its skin glands. Related toxins have been obtained from other species of the animal.

These toad poisons in their physiological action resemble the cardio-toxic glucosides, and one of them has long been used in China as the drug Ch'an Su. Bufotoxin on hydrolysis yields arginine, suberic acid, and a toxic steroid, *bufotalin*, $C_{26}H_{36}O_6$.

Provitamin Steroids

In 1927, it was shown by Rosenheim, Pohl and Windaus that ultra-violet irradiation of pure ergosterol produced a series of isomeric steroids ($C_{28}H_{44}O$), one member of which was highly anti-rachitic and had the calcium-controlling, or *calcio-kinetic*, properties of the natural vitamin D obtained from liver oils.

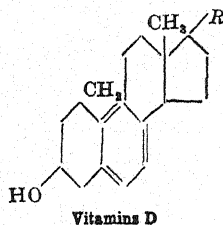
Ergosterol \rightarrow lumisterol \rightarrow tachysterol \rightarrow calciferol \rightarrow toxisterol.

Calciferol is the anti-rachitic steroid obtained artificially. It was first regarded as being identical with vitamin D, and ergosterol was accepted as being the natural provitamin. However, in 1934, Waddell found that both irradiated cholesterol and cod-liver oil concentrates were more effective than calciferol, when given in equivalent dosage to rachitic chickens, and now it is known that several different vitamins of the D type exist, including D, the anti-rachitic factor in natural liver oils, D_2 , or calciferol, and

D_3 , the factor obtained from 7-dehydrocholesterol, which is its provitamin in crude cholesterol.

Provitamins D.—The precursors of the D vitamins all appear to be steroids characterised by $—OH$ in a *normal* position at 3, and double-bonds at 5 : 6 and 7 : 8, in ring II.

When the provitamin is activated by light or chemical reagents, the 9–10 saturated linkage in Ring II is opened, and the $—CH_3$ group at 10 is dehydrogenated to $=CH_2$. Differences in the vitamins are due to the type of side-chain at 17 in the parent steroid,

 <p style="text-align: center;">Vitamins D</p>	CH ₃	R
	CH ₃	
Side-chain R.		
D_2	$—CH(CH_3) \cdot CH : CH \cdot CH(CH_3) \cdot CH(CH_3)_2$	
D_3	$—CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH_2 \cdot CH(CH_3)_2$	
D_4	$—CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH(CH_3) \cdot CH(CH_3)_2$	
D_5	$—CH(CH_3) \cdot CH_2 \cdot CH_2 \cdot CH(C_2H_5) \cdot CH(CH_3)_2$	

In D_3 the cholesterol nucleus has been *dehydrogenated* to form a double-bond at 7 : 8.

D_4 is got from 22-dihydroergosterol, in which the side-chain is *hydrogenated*, saturating the 22–23 linkage.

D_5 is got from 7-dehydrositosterol. Several other similar substances with vitamin D potency have been prepared.

The term D is usually applied to the natural vitamin found in liver oils ; it appears to be a mixture of D_2 and D_3 .

The Sex Hormones

Specific compounds elaborated in the gonad tissue of vertebrates are necessary for the mature development and functional maintenance of the genetically determined sexual type. These compounds are classed as *gynæcogens*, or female hormones, and *androgens*, or male hormones. Gynæcogens are divided into (i.) the *cestrone* group of follicular hormones, and (ii.) the hormone of the corpus luteum. Androgens are represented by the androsterone group of hormones. All these compounds are steroid in character, and they or their precursors have been detected in a variety of animal and plant materials. For example, *cestrone*, the follicular hormone is manufactured chiefly in the ovarian follicle, and is a constant constituent of the urine during pregnancy, but it has been isolated also from male urine, and from the oil of the palm tree nut. In 1930, Marrian showed that a variety of hormones closely related to *cestrone* could be separated from both male and female urine, and it is now recognised that both gynæcogens and androgens are present in both sexes.

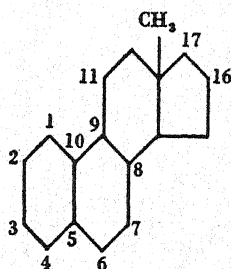
As Elderfield has observed, the classification of these factors as male hormones and female hormones is misleading. The biological effects of a particular hormone are not restricted to the reproductive organs of one sex, and the same compound may display the dual action of both male and female hormones. Provisionally, a male hormone is described as having the essential property of promoting growth of the secondary sexual structures in the male, such as the comb and plumage of capons and the seminal vesicles of castrated male rats. A female hormone has the property of promoting the œstrus cycle and uterine enlargement.

The work of Dodds and his colleagues has shown that female hormones need not necessarily be steroid in character. A stilbene derivative has been obtained, diethyl stilbœstrol, that has nearly three times the potency of the natural hormone, œstrone (p. 496).

Female Sex Hormones

The Follicular Hormone.—This hormone occurs in the urine of pregnancy in four forms: (1) *œstrone*, $C_{18}H_{22}O_2$, (2) *œstriol*, $C_{18}H_{24}O_3$, (3) *equilin*, $C_{18}H_{20}O_2$, and (4) *equilenin*, $C_{18}H_{18}O_2$; and also occurs in the ovary as (5) *œstradiol*, $C_{18}H_{24}O_2$. All these compounds are 3-hydroxy-13-methyl-derivatives of the *cyclopentanophenanthrene* nucleus, and carry an oxygen group or a hydroxyl group in position 17. They are excreted in the urine chiefly as esters of glycuronic acid, in which form they are of low activity. These compounds are typical **œstrogens**.

Unlike the other steroids, the follicular or œstrogenic hormones, or œstrins, have only one methyl group in the nucleus; it is attached at C 13.



œstrane Nucleus.

Hormone.	Double-bonds.	Groups.
œstrone .	1 : 2, 3 : 4, 5 : 10	OH, 3; 0, 17
œstradiol .	1 : 2, 3 : 4, 5 : 10	OH, 3, 17
œstriol .	1 : 2, 3 : 4, 5 : 10	OH, 3, 16, 17
Equilin .	1 : 2, 3 : 4, 5 : 10, 7 : 8	OH, 3; 0, 17
Equilenin .	1 : 2, 3 : 4, 5 : 10, 6 : 7, 8 : 9	OH, 3; 0, 17

These hormones are colourless crystallisable compounds of very low solubility in water. œstrone can be got from the dehydration of œstriol by heating with $KHSO_4$, and, on reduction, yields œstradiol, the most active of the hormones, and the form in which it occurs in the ovary.

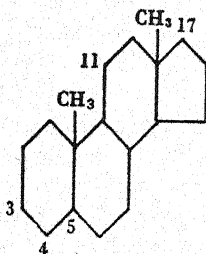
The Corpus Luteum Hormone.—Only one form is known to exist, *progesterone* (progestin, luteosterone), $C_{21}H_{30}O_2$, which has been obtained from the corpus luteum and from urine of pregnancy, where it is accompanied by inactive reduction derivatives, the alcohols, *pregnanediol*, $C_{21}H_{32}O_2$, and *allo-pregnanediol*. By degradation of the side-chain in stigmasterol, Butenandt has obtained progesterone, thus proving the steroid character of the hormone.

Progesterone is a 17-acetyl ($CH_3.CO-$) derivative of a steroid nucleus carrying a methyl group at 10 and 13, and an oxygen at 3, and having a double-bond at 4 : 5 (p. 499).

Androgens

The Testicular Hormones.—From male urine, (1) *androsterone*, and (2) *dehydro-isoandrosterone*, can be isolated; *testosterone* (3) has been obtained from testicular extracts, and has been prepared, also, from cholesterol. *Androstandiol* (4) has been obtained by reduction of androsterone; it is the diol corresponding to oestradiol, and is about three times as potent as the parent androsterone, but, unlike oestradiol, it has not yet been isolated from natural sources. The androgens resemble the gynæcogens in general structure, but differ in their degree of saturation. Testosterone is the most powerful. Chemically, the androgens are simple oxy or hydroxy derivatives of a parent steroid, *androstande*.

All three testicular hormones, and the androgen, adrenosterone, from adrenal cortex, are 10 : 13 dimethyl steroids..



Androstane Nucleus.

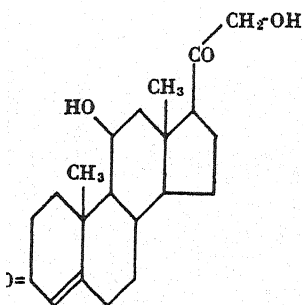
Hormone.	Double-bonds.	Side-groups.
Androsterone	none	<i>epi</i> -H at 3, : O at 17
Dehydro- <i>iso</i> androsterone	4 : 5	<i>normal</i> -H at 3, : O at 17
Testosterone	4 : 5	: O at 3, —OH at 17
Adrenosterone	4 : 5	: O at 3, 11, 17

These hormones, with the exception of testosterone, have an oxygen attached at C17, and are sometimes described as the 17-ketosteroids; this grouping enables them to be detected and estimated in urinary and other extracts by means of Zimmermann's reaction.

The Adrenal Cortex Hormones

The adrenal cortex is rich in sterols, and its internal secretion appears to be steroid in character. The cortin complex, originally obtained in crystalline form by Kendall, Reichstein and their

colleagues, has now been resolved into a mixture of active and inactive compounds, some of which contain the C_{19} nucleus. The term *corticosterone* is applied to the compound, $C_{21}H_{30}O_4$, which is reported to have the biological action of the cortical hormone. A steroid, adrenosterone, with weak androgenic properties has also been isolated from the lipid fraction of the cortex (p. 476)



Corticosterone.

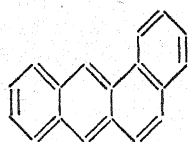
Hormone.	Double-bonds.	Side-chains.
Desoxycorticosterone	4 : 5	: O at 3, —CO.CH ₂ .OH at 17
Corticosterone	4 : 5	: O at 3, —OH at 11, —CO.CH ₂ .OH at 17

Replacement of OH at 11 by H converts corticosterone into 11-desoxycorticosterone, a product ten times more effective in prolonging the life of the decorticated rat (p. 475). Replacement of —OH in the —CO.CH₂.OH side-chain converts desoxycorticosterone into progesterone, which, though it has no cortical activity, may be a related intermediate in general steroid metabolism.

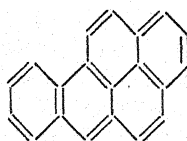
Carcinogenic Hydrocarbons

In 1915, Yamagiwa showed that application of tar was capable of evoking malignant changes in the skin of rodents. The production of these skin cancers was verified by many workers, although it was realised that animals differed in their susceptibility and tars differed in their carcinogenic property. In 1924, Kennaway obtained and isolated active carcinogens from the mixture of tars produced when pure acetylene or pure isoprene is heated with hydrogen, thus showing that the agent was a hydrocarbon, and not a contaminant. The synthetic and natural carcinogenic tars resembled one another in the possession of an intense fluorescent spectrum, which suggested the presence of polycyclic nuclei.

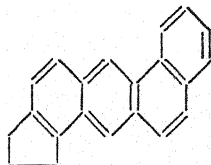
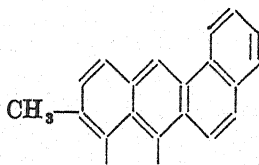
A number of such compounds were prepared and examined, and at least four were found to possess unmistakable carcinogenic properties. They are: (1) 1 : 2-benzanthrene, (2) 1 : 2-benzpyrene, (3) 5 : 6-cyclo-pentano-1 : 2-benzanthrene, and (4) methyl colanthrene, which was obtained from desoxycholic acid, and is very active.



1 : 2-Benzanthrene.



3 : 4-Benzpyrene.

5 : 6-cyclo-Pentano-
1 : 2-benzanthrene.

20-Methyl colanthrene.

Now, although none of these compounds is a true steroid, they all contain polycyclic nuclei, and, furthermore, the fact that methyl colanthrene has been prepared from a naturally occurring bile acid suggests that they must be considered as possible types of compounds produced in abnormal sterol metabolism.

9 : 10-dimethyl benzanthrene is a powerful carcinogen, and its local application may induce a skin cancer within thirty-five days.

Two at least of the synthetic carcinogens, namely, 1 : 2-benzpyrene and 5 : 6-cyclo-pentano-1 : 2-benzanthrene are also active oestrogens.

Carcinogenesis is now recognised as a metabolic perversion evoked by many apparently unrelated compounds and agencies, including prolonged exposure to ultra-violet irradiation, X-rays and radio-active substances. Carcinogenic compounds may have two types of action :—

(1) *Local*. By injuring cell nuclei, and thus inhibiting normal growth, or by evoking a mutation that leads to a new race of cells. An industrial cancer, first recognised in the late eighteenth century as a disease of occupation among chimney sweeps, has been traced to prolonged exposure to soot, the active agent, later isolated from coal tar, being 3 : 4-benzpyrene.

(2) *Distant*. Continued exposure to aniline or β -naphthylamine, under industrial conditions, may lead to bladder tumours. The incidence of some animal cancers can be increased by excessive and prolonged administration of sex hormones. Conversely, administration of *stilboestrol*, the artificial oestrogen discovered by Dodds, may check cancers of the prostate by its indirect inhibiting effect on the output of androgens by the testicle. These distant effects are distinct from local actions. Thus, *p*-dimethyl amino azo

benzene, $(\text{CH}_3)_2\text{N} \cdot \text{C}_6\text{H}_4 \cdot \text{N} : \text{N} \cdot \text{C}_6\text{H}_5$, has no obvious action when applied directly to the liver, but when given in small amounts daily in the diet results in a cancer of the liver; the compound was formerly used, for colouring foodstuffs, under the name of "butter yellow."

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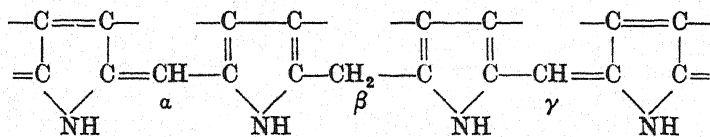
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CHAPTER 12

BIOLOGICAL PIGMENTS : PYRROLE DERIVATIVES, CAROTINOIDS, FLAVINS, MELANINS

A. PYRROLE DERIVATIVES

THE red world of the animal and the green world of the plant owe their colour to the presence of pigments derived from a simple heterocyclic compound, pyrrole, $C_4H_4.NH$, which occurs in tetrapyrrole groups of four units. Two great classes of these pigments exist: the *linear tetrapyrroles*, represented by the bile pigments; and the *cyclic tetrapyrroles*, or porphyrins, found in the blood pigments and in chlorophyll.



Tetrapyrrole skeleton.

Natural Linear Tetrapyrroles, or Bilans

Name.	Formula.	Source.
Bilirubin	$C_{33}H_{36}O_6N_4$	Bile
Biliverdin (Dehydrobilirubin)	$C_{33}H_{34}O_6N_4$	Bile
Mesobilinogen	$C_{33}H_{44}O_6N_4$	Reduced bilirubin
Urobilinogen	$C_{33}H_{46}O_6N_4$	Urine
Mesobilin (Urobilin IX α)	$C_{33}H_{42}O_6N_4$	Urine
Urobilin (stercobilin)	$C_{33}H_{46}O_6N_4$	Urine, Intestinal contents
Uteroverdin	$C_{33}H_{34}O_6N_4$	Dog placenta
Bilipurpurin (phylloerythrin)	$C_{34}H_{36}O_6N_4$	Bile

The hepatic secretion of man and other animals is deeply coloured owing to the presence of pigments, notably *bilirubin* and *biliverdin*. Bilirubin is the chief pigment in human bile, to which it imparts a golden-yellow colour. Biliverdin, an oxidation derivative of bilirubin, is found in herbivora and other animals, and imparts an emerald green colour to the bile. When both pigments are present,

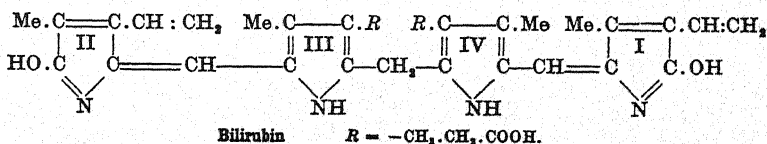
as in ox bile, the colour may vary from brown to green. These pigments are waste products derived from hæmoglobin.

Bilirubin, $C_{33}H_{36}O_6N_4$, occurs in bile as a soluble bilirubinate; in biliary calculi it is present as an insoluble calcium salt. It can be prepared from evaporated bile residues or powdered gallstones by the following method of successive extraction: (i.) ether, (ii.) hot water, (iii.) 10 per cent. acetic acid, (iv) alcohol, and (v.) hot glacial acetic acid. By this process the following substances are removed: sterols, bile salts, biliverdin, and inorganic salts. The residue is dried and extracted with hot chloroform, which removes the bilirubin. On cooling, bilirubin crystallises out.

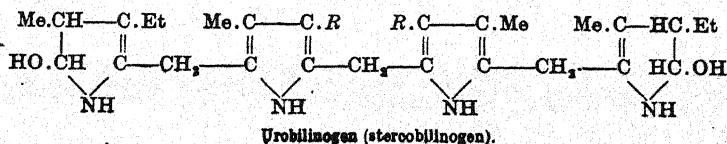
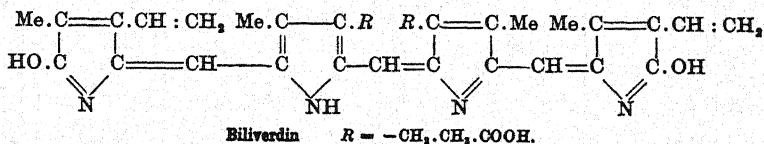
Bilirubin is a red-brown tetrapyrrole, insoluble in water, dilute acids, and the common fat-solvents. It is soluble in hot chloroform and in alkalis. The solutions show no characteristic absorption spectrum.

Structure.—Mild oxidation converts bilirubin into the green pigment, *biliverdin*; more powerful oxidants resolve it into four pyrrole units. Bilirubin does not combine with metals to form porphyrans, thus suggesting that the formula is an open chain of pyrrole units, not a closed tetrapyrrole or porphyrin.

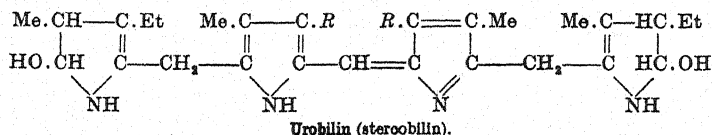
When bilirubin is reduced by sodium amalgam it is converted to a colourless derivative, mesobilinogen, which is reoxidised by atmospheric oxygen to a yellow compound, *mesobilin*, that resembles *urobilin* (stercobilin), the waste pigment present in urine and intestinal contents.



By reduction of the two vinyl groups $-\text{CH:CH}_2$, to ethyl groups, Et, $-\text{CH}_2.\text{CH}_3$, mesobilin is formed from bilirubin.



Biliverdin, or dehydrobilirubin, is derived from bilirubin by loss of two hydrogen atoms in the region of the central linkage of the tetrapyrrole chain. It is formed spontaneously when bilirubin is oxidised by exposure to air in alkaline solution, or treated with hydrogen peroxide. The pigment is precipitated by acidification, and any unchanged bilirubin removed by chloroform extraction. Biliverdin is a dark green amorphous solid, insoluble in water, ether or chloroform. It dissolves in alkalis to form salts, and is freely soluble in glacial acetic acid. These solutions have a bright green colour, but no characteristic visible spectrum. Biliverdin is the precursor of bilirubin in the formation of bile pigment from hæmoglobin.



Urobilin, or stercobilin, is a brown pigment derived from bilirubin by bacterial reduction in the intestine. It is reabsorbed into the blood, and reduced still further to the colourless chromogen, *urobilinogen*, in which form it is excreted in the urine. Urobilin is precipitated from urine by saturation with ammonium sulphate and extraction with alcohol. In solution, it shows a distinct absorption band in the region $b - F$ ($486 \text{ m}\mu - 508 \text{ m}\mu$). Addition of zinc chloride or acetate to the neutral solution causes a green fluorescence and the appearance of an additional absorption band near the b line. Faecal or urinary urobilin (stercobilin) is strongly laevorotatory, and thus differs from the artificial mesobilin, or urobilin IX α .

Urobilinogen, the colourless precursor of urobilin, is a normal constituent of fresh urine, and may be greatly increased in amount by conditions of intestinal stasis and increased intestinal putrefaction. When urine is exposed to air, or treated with mild oxidising agents, urobilinogen is converted to urobilin, and the colour of the urine darkens. Like urobilin, it is precipitated by saturation with ammonium sulphate, but, unlike urobilin, it is soluble in ether, and thus may be extracted. When pure, urobilinogen is a colourless crystalline solid. In solution it shows no absorption bands and no fluorescence on addition of zinc salts.

Urobilinogen, unlike urobilin, gives an immediate red colour with Ehrlich's aldehyde reagent in acid solution (p. 165).

Urobilin and urobilinogen do not usually occur free in urine, but in some loosely combined forms. It is possible that *urochrome*, the characteristic yellow pigment of urine, is a urobilin derivative.

Free urobilin and urobilinogen are excreted in febrile and other conditions accompanied by rapid loss of protein, and in the recovery stage of obstructive jaundice.

Natural Cyclic Tetrapyrroles, or Porphyrins

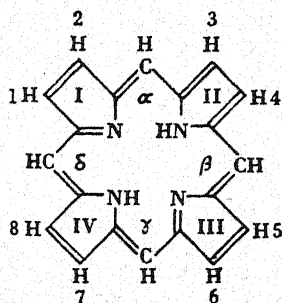
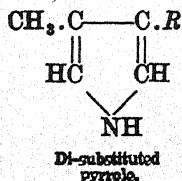
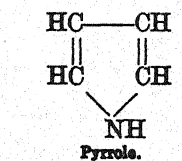
Porphyrins are widely distributed pigments of high stability. The nitrogen in the tetrapyrrole cluster readily combines with metals, such as Fe, Cu, Mg, and Mn, to form metallo-porphyrins, or *porphyrans*, having catalytic properties.

Porphyrin proteins, such as hæmoglobin, participate in oxygen transport; others, such as cytochrome, are concerned in the oxidation mechanisms of all living tissues.

Name.	Formula.	Source.
Protoporphyrin . . .	$C_{34}H_{34}O_4N_4$	Hæmoglobin
Hæmatoporphyrin . . .	$C_{34}H_{34}O_6N_4$	Hæmoglobin
Ætioporphyrin I. . . .	$C_{32}H_{32}N_4$	Chlorophyll
Ætioporphyrin III. . . .	$C_{32}H_{32}N_4$	Hæmoglobin
Coproporphyrin	$C_{36}H_{36}O_6N_4$	Urine, yeast
Uroporphyrin	$C_{38}H_{38}O_{10}N_4$	Urine, Turacou feathers

General Structure of the Porphyrins

Knowledge of the porphyrins has been reached by the convergence of work on chlorophylls, chiefly by the Willstätter school, and on the blood pigments, by Hans Fischer; and important contributions have been made by Stoll, Conant, Marchlewski, Kuhn, and Küster. The properties of the porphyrins as a class suggests a ring structure composed of four stable units. On oxidation of a porphyrin, these units are obtained in the form of di-substituted pyrroles, one substituent of which is always a methyl group.



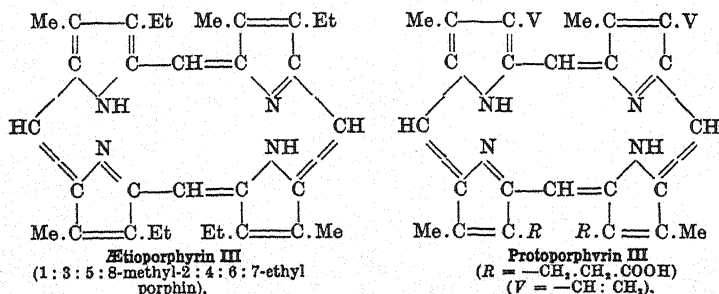
Porphin, $C_{20}H_{14}N_4$
(Parent nucleus of porphyrins)

The pyrrole units are united by $-\text{CH}=\text{}$ linkages, the positions of the imino-hydrogen atoms and double bonds in the porphyrin formula being arbitrary.

Owing to the possibility of substitution in any of the positions 1-8, many isomers of each porphyrin are possible. If four methyl-ethyl pyrroles are united, an *ætioporphyrin* is produced, of which, two isomers, denoted Series I and Series III, occur in nature, out of the possible four.

Protoporphyrin is built up from two methyl-propionic acid pyrroles and two methyl-vinyl pyrroles. Fifteen isomers are possible, but only one has been found in nature. It is homologous with *ætioporphyrin* III.

Protoporphyrin is the most important natural porphyrin, and its iron complex, in union with protein, appears as hæmoglobin, myoglobin, catalase, cytochrome, and other respiratory pigments.



Ætioporphyrin III is obtained from hæmoglobin, and has been synthesised by Fischer. It was formerly thought to be identical with the porphyrin (*phyto-ætioporphyrin*) obtained from chlorophyll, but, although closely related, the compounds differ in their substituent groups, and more than one type of phyto-*ætioporphyrin* has been prepared.

Two coproporphyrins and two uroporphyrins occur naturally.

Uroporphyrin I, 1: 3: 5: 7-*M*-2: 4: 6: 8-*S*-porphyrin; where $M = -\text{CH}_3$, and $S = -\text{CH}_2\text{CH}:(\text{COOH})_2$, occurs, in traces, in urine (porphyrinuria), and as a purple copper derivative in *turacin*, the pigment from the wing feathers of African birds of the genus *Turacus*. *Uroporphyrin* III is excreted in urine in *porphyria* (p. 466).

Protein Porphyrins

The outstanding porphyrin compounds found in the animal body are: (i) the *hæmochromes*, or oxygen-transport pigments of the blood, and (ii) the *cytochromes*, or respiratory pigments of the tissues.

The hæmochromes include the iron-containing chromoproteins, hæmoglobin, erythrocrucorin, chlorocrucorin and hæmoerythrin, and the copper-containing chromoprotein, hæmocyanin. The cytochromes are four in type, and are found in all aerobic cells.

THE HÆMOCHROMES, OR BLOOD PIGMENTS

	Hæmoglobin	Erythrocrucorin	Chlorocrucorin	Hæmocyanin	Hæmoerythrin
<i>Distribution</i>	Vertebrates	Many invertebrates	Polychate worms	Molluscs Arthropods	Gephyreans
<i>Colour</i>	Red	Red	Green	Blue	Red
<i>Prosthetic group</i>	Hæm	Hæm	Hæm	Thio-peptide	
<i>Metal</i>	Fe	Fe	Fe	Cu	Fe
<i>Location</i>	Corpuscles, Muscle	Corpuscles	Plasma	Plasma	Corpuscles or plasma
<i>M. Wt.</i>	68,000	17,000 to 5×10^4	300,000 to 5×10^4	300,000 to 5×10^4	17,000 to 68,000

Hæmoglobin, the dominant pigment of animal life, occurs in the red cells of all vertebrates. It is a chromoprotein, made up of 94 per cent. of globin and 4-5 per cent. of the porphyrin, hæm, the residue being mostly lipid material. Not only does the hæmoglobin differ in different species but there is evidence for the presence of more than one form of hæmoglobin in the same animal, and obscure racial relationships may be traced this way. These species-differences are ascribed to differences in the globin component of the molecule. Crystalline hæmoglobin can be obtained directly from the blood of horses, dogs, and rats, after "laking," or hæmolysis of the red cells by addition of water or ether.

Derivatives of hæmoglobin.

A. *Addition compounds*: oxyhæmoglobin, nitroxyhæmoglobin, carbonylhæmoglobin, sulphæmoglobin.

B. *Decomposition products*: hæmochromogen, hæmin, hæm, hæma in, hæmatoporphyrin.

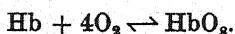
Oxyhæmoglobin, HbO_2 , a scarlet compound of hæmoglobin containing two atoms of displaceable oxygen for each atom of iron in the molecule. It forms spontaneously when a solution of hæmoglobin is shaken with air, and it is responsible for the transport of oxygen in the blood stream.

In concentrations of 1:1,000 to 1:10,000, oxyhæmoglobin has a characteristic spectrum with two absorption bands between the D and the E lines. The α -band is the narrower and more distinct, and lies on the D line, the middle of the band being at $579 \text{ m}\mu$. The

middle of the β -band is about 542 $m\mu$.

Oxyhæmoglobin has a third band (Soret's band), located in the ultra-violet region of the spectrum between G and H, its centre being at 415 $m\mu$. When oxyhæmoglobin is reduced, this band is displaced towards the visible spectrum. On exposure to low atmospheric or oxygen pressure, or when treated with reducing agents such as hydroxylamine, sodium hydrosulphite, ammonium sulphide, oxyhæmoglobin is readily reconverted into hæmoglobin, which shows a single diffuse band between the D and E lines.

The Interaction between Hæmoglobin and Oxygen.—Crystallised hæmoglobin, irrespective of its source, contains 0.335 per cent. of iron, which is in the ferrous form. One gram of hæmoglobin combines with 1.34 ml. of oxygen, at 0° C. and 760 mm. Hg (atmospheric pressure), a ratio corresponding to two atoms of O for each atom of Fe. The union is molecular; oxygen unites and dissociates in the form of O_2 , without oxidising the Fe. Assuming that a molecule of Hb contains only one atom of Fe, the molecular weight is approximately 16,700. But direct determinations of sedimentation rate and osmotic pressure indicate a value of about 68,000, which is four times as large. Unless this is due to molecular aggregation in solution, the formula for oxyhæmoglobin is HbO_2 , and the reversible equation is :



Methæmoglobin, HbO or $HbOH$, is formed when hæmoglobin is oxidised in alkaline solution with permanganate or peroxide, or when potassium ferricyanide is added to oxyhæmoglobin. The colour of the solution turns chocolate-brown, and the characteristic spectrum of methæmoglobin develops. In acid solution there is an absorption band towards the red end of the spectrum, between C and D, its centre being about 634 $m\mu$. In alkaline solution, two absorption bands are seen, resembling those of oxyhæmoglobin but differing in that the β -band is sharper than the α -band. Methæmoglobin is formed also by the action of nitrobenzene, pyrogallol, acetanilide, and other phenols and amines, and may appear in the urine accompanying the hæmaturia due to poisoning by chlorate, nitrate, or phenols.

In methæmoglobin, the iron has been oxidised to the ferric form, and the pigment is no longer able to take part in oxygen transport as it does not yield up its oxygen on exposure to low pressures. By the action of reducing agents, or by intravenous injections of glucose into the circulation, methæmoglobin is converted into hæmoglobin, and thus restored to physiological utility.

Carbonylhæmoglobin, or carbon monoxide hæmoglobin, HbCO , is formed by the action of CO on hæmoglobin or oxyhæmoglobin, from which it displaces the oxygen. The spectrum shows two absorption bands resembling those of oxyhæmoglobin but shifted more towards the violet end; the centre of the α -band being at $570 \text{ m}\mu$, and the β -band at $535 \text{ m}\mu$.

Distinction between oxy- and carbonylhæmoglobin :—

(1) Carbonylhæmoglobin is more pink or "cherry red" than oxyhæmoglobin solutions of the same concentration, and on dilution the colour of the carbonyl compound remains pink, while the oxyhæmoglobin turns yellowish.

(2) On treatment with ammonium sulphide or similar reducing agents, carbonylhæmoglobin is unchanged, while oxyhæmoglobin is converted to hæmoglobin. This test can best be followed by means of a spectroscope. By the action of a strong reducing agent, such as sodium hydrosulphite, aided by heat, carbonylhæmoglobin can be converted into hæmoglobin.

(3) By means of the reversion spectroscope, carbonylhæmoglobin can be detected and estimated in presence of oxyhæmoglobin.

The term *carbonylhæmoglobin* is preferable to the usual *carboxyhæmoglobin*, which implies the presence of a carboxy, or $-\text{CO}_2$ group, rather than the $-\text{CO}$, or carbonyl group, actually present.

DECOMPOSITION PRODUCTS OF HÆMOGLOBIN

Hæmochromogen is a chromoprotein formed by the action of alkalis and reducing agents on hæmoglobin. When a dilute solution of blood is warmed with an alkali the colour changes from red to greenish-brown. This is due to (1) formation of methæmoglobin, and (2) its decomposition into the protein *globin* and the iron porphyrin *hæmatin* or *methæm*. At the same time, the alkali denatures the liberated protein. If now a reducing agent be added, such as sodium hydrosulphite, the hæmatin is converted to reduced hæmatin or *hæm*. Hæm rapidly recombines with the denatured globin to form a new chromoprotein *hæmochromogen*.

In alkaline solution this hæmochromogen has a bright carmine colour, and shows two bands somewhat like those of oxyhæmoglobin but nearer the violet end of the spectrum. The α -band is the darker and narrower, and its centre is at $556 \text{ m}\mu$, almost midway between the D and the E lines. The β -band covers the E and the δ lines, and has its centre about $528 \text{ m}\mu$. Since the spectrum of hæmochromogen can be detected in dilutions at which the spectra of oxy- and carboxyhæmoglobin are invisible, its formation is sometimes used as a test for traces of blood.

The pigment can be distinguished from oxyhæmoglobin by its stability to reducing agents.

Hæmochromogens are also formed readily by union of hæm with pyridine, nicotine, and similar bases.

Takayama's Test.—Spread a very small drop of blood on a slide so as to form a film. Let dry in air. Add 2–4 drops of the reagent, and cover with a slip to prevent evaporation. After 10–15 minutes, examine microscopically for the small pink crystals of pyridine hæmochromogen.

The reagent is: pyridine, 3 ml.; glucose, 3 gm.; 2·5 per cent. NaOH, 12 ml. It is ready for use after 24 hours, and remains active for about 3 months. It is of special value in detection of blood stains.

Hæmatin, **methæm**, or oxidised hæm, $C_{34}H_{32}N_4O_4FeOH$, a base which in reduced form occurs united to protein in hæmochromogens. It is a dark, amorphous powder, insoluble in water and many organic solvents, but dissolves in alkalies or in glacial acetic acid forming solutions termed *alkaline* and *acid hæmatin*, respectively. The change of colour blood undergoes when warmed with alkalies or acids is due to the formation of the corresponding hæmatin.

When a mixture of alcohol and ether is added to dilute blood which has been acidified with a few drops of hydrochloric acid, the chromoprotein is decomposed and the liberated hæmatin passes into the alcohol-ether layer.

As ordinarily prepared, "hæmatin" is a mixture of a ferric porphyrin, *methæm*, and a ferrous porphyrin, *hæm*. Hæmoglobin, itself, is a chromoprotein formed by the union of globin and hæm, and when oxidised to oxyhæmoglobin, the hæm component is converted into a labile form, oxy-hæm. Drastic oxidisers change the ferrous oxy-hæm into the ferric methæm, as occurs when methæmoglobin is prepared.

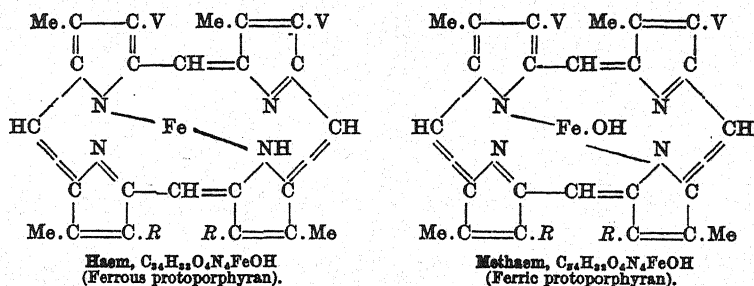
Hæmatin chloride, or **hæmin**, $C_{34}H_{32}O_4N_4FeCl$, a purple-brown crystalline salt that forms spontaneously in old blood clots, and may be accompanied by crystals of biliverdin, which formerly were termed "hæmatoidin."

The hæmin crystals are dark brown elongated rhomboids or spindles, occurring singly or grouped as crosses or rosettes. Hæmin is insoluble in water, dilute acids, and neutral organic solvents, but dissolves with decomposition in alkalies, forming hæmatin, which may be precipitated in a pure condition by acidification.

Hæmin is important chemically as it is the starting-point for the study of the hæmatoporphyrin compounds.

Structure of the Hæms.—The iron-porphyrans, or hæms, like the other porphyrans are mono-metallic derivatives of a porphyrin. In hæm the iron is ferrous (divalent) and bound by the pyrrole nitrogens, conventionally regarded as belonging to rings I and II, or I and III. In hæmatin (methæm) and in hæmatin chloride, the

iron is ferric (trivalent) and can combine with an anion, such as OH' (in methæm) or CL' (in hæmin).



Hæmatoporphyrin, $C_{34}H_{38}O_6N_4$, an iron-free derivative of hæm, is obtained by the action of strong acids on hæmoglobin, or by dissolving hæmatin chloride in glacial acetic acid saturated with HBr. After four days, the mixture is diluted and the porphyrin precipitated by exact neutralisation. Hæmatoporphyrin is a dark violet powder, almost insoluble in water, but soluble in alcohol, alkalis, and concentrated sulphuric acid. It is a di-hydroxy derivative of **Protoporphyrin**, in which each vinyl side-chain, $—CH : CH_2$, has become $—CH_2 \cdot CH_2 \cdot OH$.

The acid solution has a very distinctive pair of absorption bands, one on either side of the D line. These may be demonstrated by the addition of 1–2 drops of undiluted blood to 10 ml. of concentrated sulphuric acid, and spectroscopic examination of the resulting purple mixture.

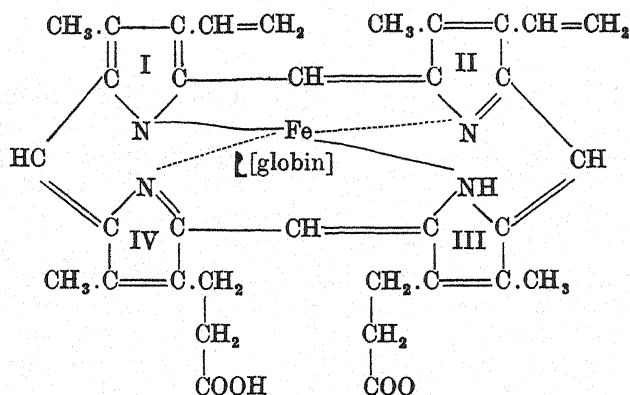
Derivatives of Protoporphyrin.—On heating with soda-lime, carbon dioxide is lost and *ætioporphyrin III* is formed. This porphyrin resembles one originally obtained from chlorophyll, and its preparation from the blood pigments is of great biochemical interest.

When protoporphyrin is oxidised in acid solution it is resolved into four pyrrole residues, the *hæmopyrroles*, from the study of which the structure of the original porphyrin has been confirmed.

The Phototoxic Properties of Hæmatoporphyrin.—Small intravenous injections of hæmatoporphyrin have no ill-effect on albino mice, rats, and guinea-pigs, as long as the animals are kept in the dark. On exposure to light, however, a severe dermatitis develops, often followed by œdema and death. The response persists for some weeks after sensitisation. It is not referable to foreign protein accompanying the injection, as animals are equally sensitive to autogenous hæmatoporphyrin.

Structure of Hæmoglobin.—Hæmoglobin is a compound of the colourless, basic protein globin and the dicarboxy acid hæm, which

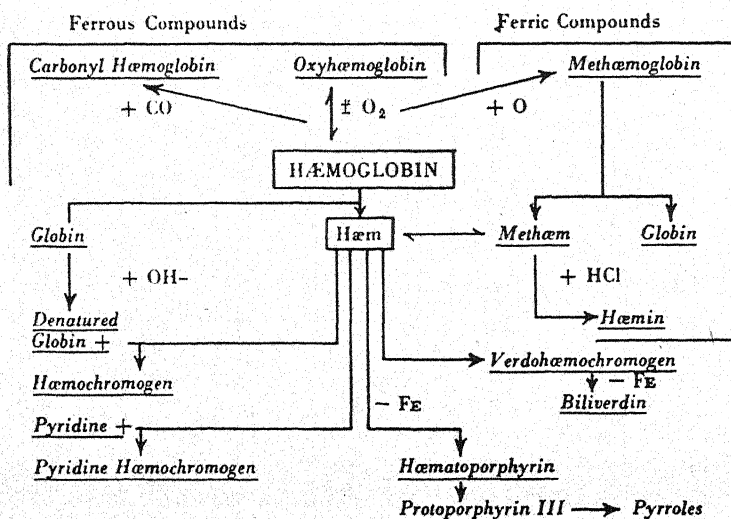
contains a carboxyl group at the end of one side-chain in rings III and IV of the parent protoporphyrin. Hæmoglobin is only stable in neutral solutions, and relatively slight degrees of acidity or alkalinity are sufficient to resolve it into globin and hæm. As the constitution of globin is not known, it is only possible to represent hæmoglobin by a semi-structural formula.

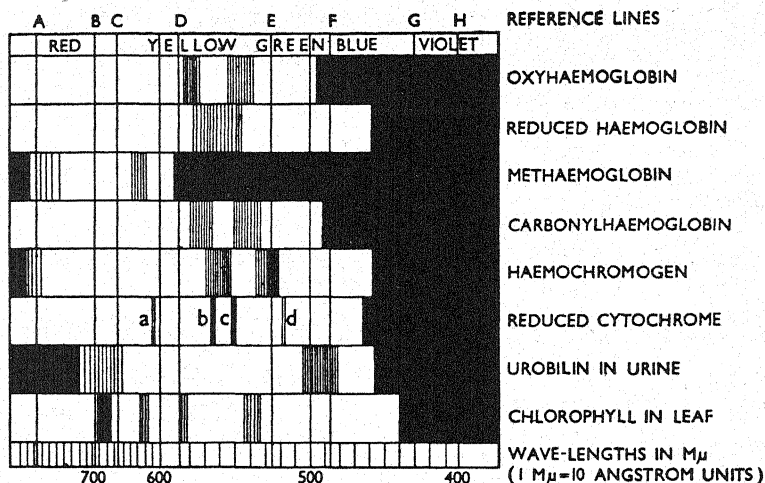


Hæmoglobin
(Globin hæmochromogen).

In oxyhæmoglobin, the additional oxygen atom is assumed to be held loosely by a residual valency to the Fe complex.

Relationship of the Hæmoglobin Derivatives





Absorption Spectra of Pyrrole Pigments.

The sodium reference lines are D, 590 $m\mu$, and D_2 , 588.9 $m\mu$.

THE CYTOCHROMES

Cytochrome.—In 1884, MacMunn observed that muscle and many other animal tissues possessed a sharply defined four-banded spectrum, which he attributed to a special pigment, *myohaematin* or *histohaematin*, so-called from its spectrographic resemblance to blood haematin. Hoppe-Seyler, the discoverer of haemochromogen, opposed MacMunn's conclusion, and the work remained neglected until, in 1925, Keilin, using an intense system of illumination which allowed the examination of thick layers of tissue, showed that histohaematin was universally distributed in all aerobic cells, and renamed the pigment *cytochrome*.

Cytochromes are present in all plant and animal tissues, and in aerobic unicellular organisms, such as yeasts and bacteria.

The spectrum shows four absorption bands, the position of which may differ slightly in cytochromes from different sources, but the general pattern of which shows a fundamental constancy. The position of maximum density of each band is: $a = 604.6 m\mu$; $b = 566.5 m\mu$; $c = 550.2 m\mu$; $d = 521.0 m\mu$. The c band being of greatest intensity is the one first sought.

Oxidation of cytochrome causes a disappearance of the characteristic spectrum, reduction, which may occur spontaneously in living tissue, causes its reappearance.

Cytochrome is a mixture of haemochromogens, a , b , and c , each

of which contribute two bands to the common spectrum: either *a*, *b* or *c*, and one component of the *d* band. A fourth, cytochrome *b*₂, has been found in yeast (Bach, 1942).

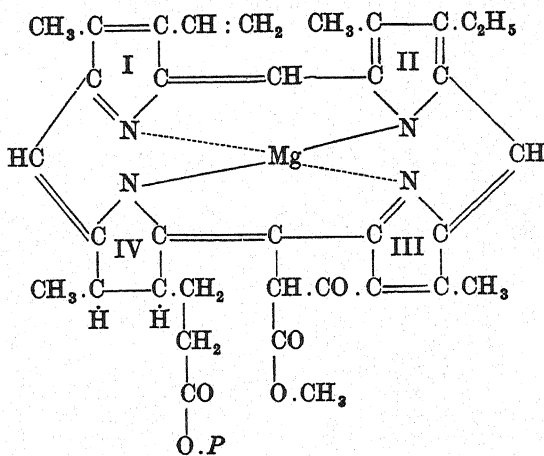
Function of Cytochrome C.—A general correspondence exists between the cytochrome content and the respiratory activity of a tissue, as measured by its oxygen consumption in presence of an oxidisable substrate. When dilute (M/100), cyanide is able to stop about 90 per cent. of the respiratory activity of most tissues, owing to its inhibitory effect on the enzyme, *cytochrome oxidase* (indophenol oxidase), which is necessary for the re-oxidation of cytochrome during the respiratory cycle. Observations such as this lead to the conclusion that cytochrome is "the main line of communication between oxygen itself and the substances which undergo oxidation in the cells" (Baldwin, 1936). The residual cyanide-stable respiratory activity is attributed to the presence of another respiratory catalyst, the "yellow enzyme" of Warburg, which differs from cytochrome in not being a hæm pigment and does not require the coaction of cytochrome oxidase. Animal cytochrome occurs chiefly in skeletal and cardiac muscle, liver and kidney.

CHLOROPHYLL

Chlorophyll, the green pigment of plant life, can be extracted easily from leaves by alcohol, acetone, ether, and similar organic solvents. The yield is about 1–2 gm. per kg. of fresh leaves, and 5–10 gm. per kg. of dried leaves. By subsequent extraction with a mixture of light petroleum and methyl alcohol, the pigment can be resolved into about 80 per cent. of chlorophyll *a* (soluble in petroleum) and about 20 per cent. of chlorophyll *b* (soluble in methyl alcohol). Both pigments are green, but they differ in their spectra in ethereal solution; chlorophyll *a* has a broad band in the red, 675–640 m μ , and a narrower band in the orange, 615–605 m μ , chlorophyll *b* has a band to the right of the C line, 655–635 m μ .

Structure.—Willstätter has shown that when chlorophyll is extracted from leaves by ethyl alcohol, an enzyme, *chlorophyllase*, present in the leaf, catalyses an alcoholysis of the pigment, splitting off phytol alcohol, C₂₀H₃₉.OH, which is replaced by a —C₂H₅ group to give a mixture of *a* and *b* chlorophyllides. By alkaline hydrolysis, these chlorophyllides are converted into chlorophyllins, which are tricarboxy acids derived from a magnesium porphyrin, *ætiophyllin*. Treatment with acid removes the magnesium, leaving an *ætioporphyrin* closely related to the protoporphyrin found in hæmoglobin and hæm pigments. A large number of intermediate degradation compounds have been obtained by Willstätter, Fischer, and their

colleagues, who conclude that chlorophyll is the methyl phytyl ester of a tricarboxy magnesium porphyrin, the third carboxyl group of which is closed to form a lactam ring.

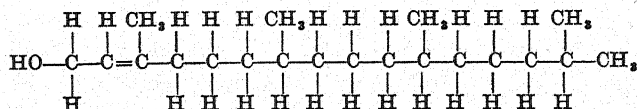


Chlorophyll *a*
(Fischer formula).

P represents the phytyl radicle, $C_{30}H_{61}$, which on hydrolysis of chlorophyll is liberated as phytyl alcohol, or phytol.

In chlorophyll *b*, the $-CH_3$ group in ring II has been oxidised to $-CHO$.

Phytol is a monohydroxy alcohol derived from the linear hydrocarbon hexadecane. Its origin and significance is unknown, but it is a probable precursor of the carotinoid pigments, and is a component of vitamin K.



Phytol, $C_{30}H_{61}OH$
(2:6; 10:14-tetramethyl- Δ^{15} , 15:16-hydroxy hexadecane).

Function of Chlorophyll.—The chief absorption band of chlorophyll, in the leaf, is in the red, about $678m\mu$, which is in the region of maximum solar energy during the day. The radiant energy thus trapped is used to effect the reduction of the absorbed and bound CO_2 , by means of H derived from H_2O .

THE SIGNIFICANCE OF THE PORPHYRINS

Two types of porphyrin structure occur naturally :

Series I, in which the four methyl groups are symmetrically set in alternate positions (1, 3, 5, 7) round the porphin nucleus.

Series III, in which the four methyls occupy positions 1, 3, 5 and 8. Series III is more common, and is represented by protoporphyrin III, *ætioporphyrin III*, and coproporphyrin III, and also occurs in hæmoglobin and chlorophylls. Transition from one series to another can only be effected by opening the stable porphin ring, which suggests that the two series have arisen independently, or at different times during biological evolution. The presence in early foetal life of uroporphyrin I, which is subsequently replaced by the series III, may be a vestigial chemical survival (Dobriner and Rhoads, 1940).

Transport of the Pyrrole Nucleus from Plants to Animals.—The porphyrin nucleus is both chemically and biologically stable, and once synthesised is not easily broken down either by plants or animals. Its history begins in the plant, where the pyrrole ring is synthesised, and appears in the amino acids tryptophane, proline and hydroxyproline, as well as in the hæm pigments and in chlorophyll. The ability of the higher animal to synthesise the pyrrole ring has been established, but it is known that one at least of the pyrrole-containing amino acids, tryptophane, is essential in animal nutrition. Consequently, it may be that the animal is dependent largely on the plant for its porphyrin units.

Chlorophyll is quantitatively an important constituent in the diet of herbivora, and a constant constituent in the human diet, and the suggestion has often been made that it is of value in nutrition.

Claims have been made by Abderhalden and others that chlorophyll has a therapeutic value in anæmia. There are, however, objections to this alleged nutritional importance. Chlorophyll is not attacked by any of the enzymes of the human alimentary tract, and its utilisation has to depend on bacterial decomposition. This may be significant in ruminants, where the parasitic factor in digestion is recognised, but is hardly so in man. It must not be forgotten that chlorophyll when it is degraded forms an important source of magnesium, in addition to providing porphyrins.

Marchlewski (1924) has shown that bilipurpurin, or cholehematin, a pigment present in the bile and biliary concretions of ruminants, is identical with *phylloerythrin*, a porphyrin pigment which he obtained by the biological degradation of chlorophyll or the acid hydrolysis of chlorophyllides. His discovery of phylloerythrin thus

leads to the conclusion that the chlorophyll molecule may enter into the metabolic processes of herbivorous animals.

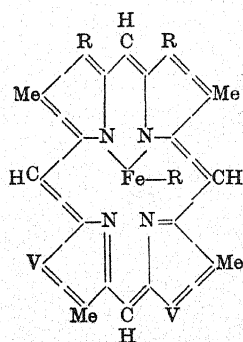
The Evolutionary Link between Plants and Animals.—When investigators first realised that all flesh is grass, as far as its pigments are concerned, it was hoped that a path of chemical evolution might be traced between the two kingdoms. Verne, for example, suggested that the hæm of animals may arise from vegetable chlorophyll, and, in consequence, the development of vertebrates was delayed until green plants had appeared in abundance. But, as Barcroft points out, the respiratory pigment cytochrome may be a much more ancient porphyrin than chlorophyll.

Yeast and bacteria, for example, grown on media entirely free from chlorophyll exhibit the spectral bands of cytochrome and hæm, which suggests that the chlorophyll has been evolved from hæm by the organism, and not hæm from chlorophyll.

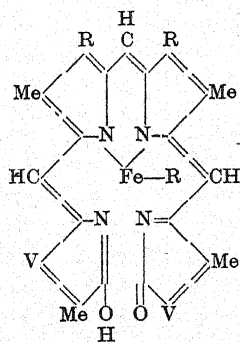
"Every detail points to something, certainly ; but generally to the wrong thing. Facts point in all directions, it seems to me, like the thousands of twigs on a tree. It's only the life of the tree that has unity and goes up—only the green blood that springs, like a fountain, at the stars."

G. K. CHESTERTON.

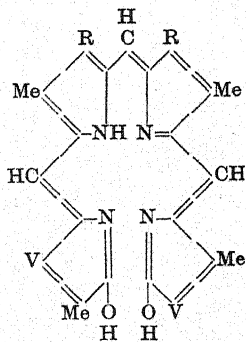
Conversion of Hæmoglobin Pigment into Bilirubin.—Lemberg has shown that oxidation of a hæmochromogen opens up the tetrapyrrole ring to form a green verdohæmochromogen, which has a linear tetrapyrrole, *verdohæmatin*, convertible into biliverdin by removal of the iron atom by acids.



Hæmochromogen.

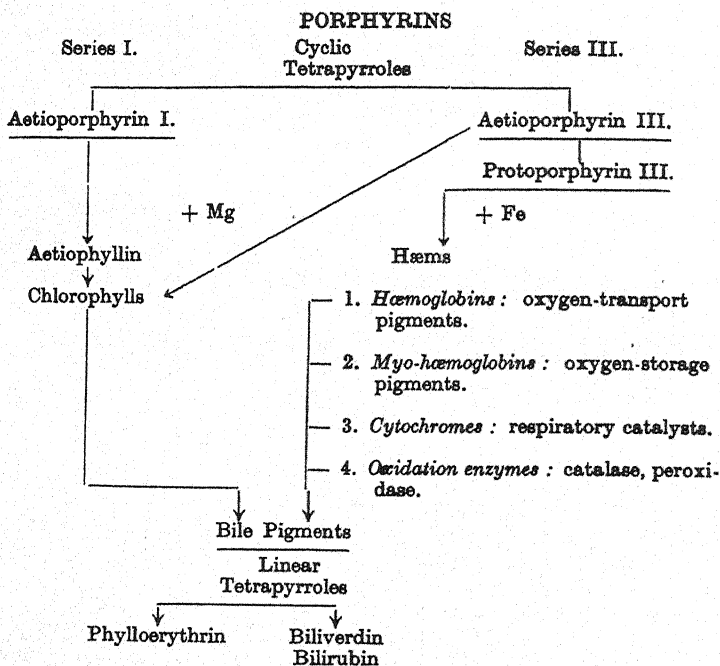


Verdohæmochromogen.



Biliverdin.

Subsequent reduction of the biliverdin forms bilirubin.



CAROTINOIDS

Carotinoids are fat-soluble hydrocarbon pigments widely distributed in low concentrations throughout the animal and plant kingdoms. They are orange or red in colour, crystallisable, freely soluble in light petroleum, chloroform and similar fat-solvents, but insoluble in water. They are easily bleached by oxidation, and give a deep blue colour in presence of concentrated sulphuric acid (the carotinoid reaction). Since they accumulate in oils and fats, they are also termed *lipochromes*. Carotinoids impart the typical colour to egg-yolk, the corpus luteum of the ovary, butter fat, liver oils, carrot, turnip, maize, tomato, "the sere and yellow leaf," and many fruits and flowers.

History.—The group name is derived from that of the first member discovered, *carotin* or carotene, which, in 1831, was isolated from carrots, and subsequently was found to occur along with another yellow pigment, *xanthophyll*, in all chlorophyll-containing foliage. Between 1906 and 1914, Willstätter isolated nearly a dozen different carotinoids, and Palmer showed that the pigment of milk and butter fat is carotene derived from the diet of the cow (1922). The pigment of egg-yolk, originally termed "lutein," was

shown to be a mixture of carotene and leaf xanthophyll, the latter being now re-named *lutein*.

PLANT CAROTINOIDS

In Order of Adsorption from Petroleum Solutions

Name.	Formula.	Chief Sources.	Adsorbent.
<i>Alcohols :</i>			
Flavoxanthin . . .	$C_{40}H_{56}O_3$	Buttercup	$CaCO_3$
Violaxanthin . . .	$C_{40}H_{56}O_4$	Pansy	"
Taraxanthin . . .	$C_{40}H_{56}O_4$	Dandelion	"
Lutein . . .	$C_{40}H_{56}O_2$	Green leaves	"
Zeaxanthin . . .	$C_{40}H_{56}O_2$	Maize	"
<i>Ketone :</i>			
Rhodoxanthin . . .	$C_{40}H_{50}O_2$	Rose fruit	"
<i>Hydrocarbons :</i>			
γ -Carotene . . .	$C_{40}H_{56}$	Carrot	$Al(OH)_3$
β -Carotene . . .	$C_{40}H_{56}$	Carrot	"
α -Carotene . . .	$C_{40}H_{56}$	Carrot	"

Carotene, $C_{40}H_{56}$.—Fresh carrot has 0.005 to 0.01 per cent. of carotene, and fresh grass contains about 0.01 per cent. of carotene and 0.02 per cent. of lutein (xanthophyll). The pigment is easily obtained by extracting dried carrot scrapings or dried leaves with light petroleum (which does not extract the chlorophyll). The extract is evaporated in a dish at room temperature, and the residue freed from lipides by rinsing with small quantities of the solvent. The carotene fraction remains as a crop of dark red micro-crystals, which by chromatographic adsorption, can be resolved into three isomers.

α -Carotene, m.p. 175° , $[\alpha]_D = +323^\circ$, forms brilliant rhomboidal crystals, copper-red in colour.

β -Carotene, m.p. 185° , $[\alpha]_D = 0^\circ$, resembles the α -form, but is slightly less soluble. β -Carotene occurs almost pure in spinach and in red pepper (paprika). The carotene fraction from palm-nut oil contains about 60–70 per cent. of the β -form, the rest being α -carotene.

γ -Carotene is rare in higher plants, but has been found in the acid-fast bacteria. It differs from the other isomers in having only one ring in its molecule.

Lycopene, $C_{40}H_{56}$, the colouring matter of tomatoes, differs from the carotenes structurally in having a non-cyclic end-groups.

Chromatographic Analysis

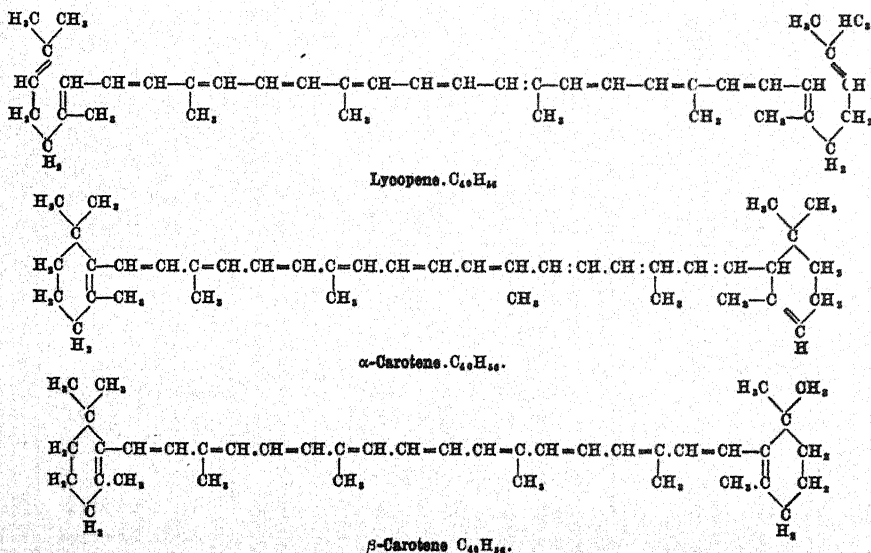
The Russian botanist, Tswett, found in 1906 that a tube packed with powdered calcium carbonate could be used to separate pig-

ments from solution in non-polar solvents, such as light petroleum. When the solution was allowed to flow down the tube, the pigments tended to be adsorbed at different stages, thus producing a striated *chromatogram*. Twenty years later, Kuhn and his colleagues applied this method to the resolution of the carotinoids, and were able to fractionate crude carotene into (i.) an optically inactive β -carotene, and (ii.) a dextro-rotatory α -carotene, the latter being adsorbed below the former. A narrow zone of pigment above the β -carotene layer proved to be a third isomer, γ -carotene, 34 mg. of which were obtained from 35 gm. of crude carotene, which, in turn was got from 300 kg., or about 6 cwt., of raw carrots.

Chromatographic adsorbability is increased by the presence of hydroxyl groups and unsaturated linkages in the adsorbent, and thus affords information about the structure of the compounds.

Structure of the Carotinoids

On reduction, lycopene was found by Karrer to accept 26 hydrogen atoms and give rise to a paraffin hydrocarbon, $C_{40}H_{52}$, which led him to conclude that the pigment is a linear compound containing 13 unsaturated linkages. Carotene, although an isomer of lycopene, was found by Zechmeister to accept only 22 atoms of hydrogen, from which he concluded that it contained two cyclic groups separated by an unsaturated chain. These groups were subsequently identified as being derived from β -ionone, a constituent of the essential oil of the violet.



The hydrocarbon carotinoids differ from each other in the arrangement of the terminal groups, both of which may be open, as in lycopene, or both closed to form 6-membered rings, as in α - and β -carotene.

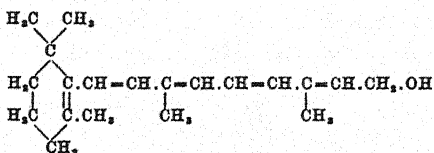
The xanthin carotinoids are ketones or alcohols derived from carotenes by substitution of H in the terminal rings.

Vitamin A

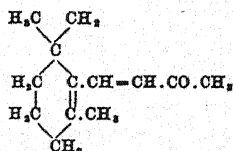
In 1919, Steenbock suggested that the fat-soluble vitamin A was related in some way to the carotinoids because of the similarity of distribution in natural sources. Inadequate knowledge of the properties of the vitamins delayed confirmation of the hypothesis, until, in 1929, von Euler showed that although carotene was not identical with vitamin A, it could replace the vitamin in the diet. Moore subsequently demonstrated that the pigment was transformed into the vitamin within the liver of the animal. Meanwhile, the carotenes had been resolved into their isomers, and it was found that β -carotene had twice the provitamin potency of α -carotene. Since the formula of vitamin A was known approximately to be $C_{20}H_{29}.OH$, it was suggested that the vitamin was hydroxy-*semi*- β -carotene, two molecules of which were derived from one molecule of β -carotene by hydrolytic fission.

α -Carotene, γ -carotene, cryptoxanthin and myxoxanthin and aphanin (from algae) each contain a *semi*- β -carotene residue, and can give rise to one molecule of vitamin A. Consequently, the six carotinoids are provitamins of A, and β -carotene is taken as the international standard for comparison (p. 272).

Structurally, vitamin A contains a β -ionone nucleus carrying a side-chain with four unsaturated linkages and a terminal primary alcohol group. A related form, vitamin A₂, $C_{21}H_{31}O$, occurs in livers of fresh-water fishes. It differs from A, in having an additional double bond in the ring. Neither vitamin has been found in plants.



Vitamin A.



β -Ionone.

Animal Carotinoids.—The carotinoids found in higher animals are usually plant carotinoids derived from the diet; the grass of the field being the principal source of the provitamins necessary for human life. Although the carotene : xanthophyll ratio in grass is 1 : 2, both cows and horses preferentially absorb the carotene to the exclusion of the xanthophyll. Birds accumulate xanthophyll

almost exclusively, and it reappears in the egg-yolk, body fat and plumage, a fact known empirically by canary breeders. Man absorbs carotene and xanthophyll with equal facility, and both pigments occur in human plasma. Milk fat, the commonest dietetic source of carotene, contains from 1 to 20 parts per million, depending on the nature of the food consumed.

Among the lower animals, carotinoids occur as special body pigments. The shells of the lobster and other crustacea contain a blue chromoprotein, which on hydrolysis by boiling liberates *astacene*, $C_{40}H_{56}O_4$, a tetra-keto- β -carotene. Astacene is the typical pigment of echinoderms, gold-fish skin and the flesh of salmon. *Violerythrin*, $C_{40}H_{56}O_6$, is the carotinoid that as an ester, *actinoerythrin*, imparts the brilliant colour to sea anemones.

Biogenesis of the Carotenoids.—According to Karrer, the alcohol *phytol*, $C_{20}H_{39}.OH$, which forms about one-third of the chlorophyll molecule, is the parent substance of the carotinoids. Desaturation of two phytol residues would in theory yield lycopene, from which the other carotinoids could arise by closure of the terminal groups to form rings. The symmetry of the carotinoid molecule suggests an origin from such a precursor.

The structural unit of both phytol and the carotinoids is *isoprene*, $H_2C=C(CH_3)-CH=CH_2$, which presumably arises during the photosynthesis of carbohydrates.

Analytical Reactions of the Carotinoids

Examine microscopically a thin section of carrot. In the cortical region minute needle-shaped orange crystals can be seen in the cells. These are stearate coloured by carotene. Cover the preparation with a glass slip, and carefully instil a drop of concentrated sulphuric acid. The crystals develop a deep blue colour.

Apply the following tests to a specimen of fish-liver oil diluted 1 : 10 with chloroform, or to a solution of carotene in chloroform.

(1) *Ferric Chloride Test.*—Addition of a few drops of 1 per cent. ferric chloride to 5 ml. of the solution produces a bright green colour owing to reduction of the iron to the ferrous state.

(2) *Sulphuric Acid Test.*—Careful addition of a couple of drops of concentrated sulphuric acid to a water-free solution of a carotinoid produces a transient blue-violet colour, which is very marked if the mixture contains vitamin A.

(3) *Antimony Trichloride Test.*—When 2–5 ml. of a chloroform solution of a carotinoid are treated with an excess of a 30 per cent. solution of antimony trichloride in chloroform a stable blue colour develops.

A similar colour is given by vitamin A (Carr-Price test), but the

vitamin blue can be distinguished by the presence of an absorption band in the region of 562–583 $m\mu$, which is not shown by any of the carotinoids. A maximum absorption band is shown by vitamin A_1 at 617 $m\mu$; and by vitamin A_2 at 693 $m\mu$.

LYOCHROMES, OR FLAVINS

It has been known for many years that animal tissues display a greenish fluorescence on exposure to ultra-violet radiation. In 1929, Ellinger, during an exploration by means of his "intra-vital microscope," observed that the fluorescent pigment was concentrated chiefly in the liver and upper renal tubule epithelium of all species of animals examined. Using the fluorescence as a guide, he succeeded in extracting and purifying the compound by absorption on Fuller's earth, and subsequent elution with aqueous pyridine. Milk whey proved to be a good source of the pigment, which was eventually obtained in crystalline form, and was termed a *lyochrome*, since it was water-soluble, thus differing from the fat-soluble lipochromes, or carotinoids.

Meanwhile, two groups of investigators working along different lines had also recognised the existence of this new type of pigment. Warburg and Christian had shown that their "yellow ferment" or respiratory catalyst, originally obtained from brewer's yeast, on hydrolysis liberated a lyochrome apparently identical with one obtained by Ellinger; and Kuhn, during a study of the vitamin B complex, found that one member, vitamin B_{12} , was itself a lyochrome.

In addition, lyochromes were obtained from a variety of animal and plant sources, and were named by affixing the termination *flavin*, to indicate the yellow colour of the pigment: *Lactoflavin*, from whey; *ovoflavin*, from egg-white; *hepatoflavin*, from liver; *renoflavin*, from kidney; *uroflavin*, from urine; *maltoflavin*, from malt; and *zymoflavin*, from yeast.

Definition.—Lyochromes are nitrogenous pigments derived from *iso*-alloxazine, and characterised by: (1) solubility in water and insolubility in fat-solvents; (2) yellow colour in solution, and orange-red colour in crystalline form; (3) greenish-yellow fluorescence in neutral aqueous solutions, the fluorescence being extinguished by addition of acid or alkali; (4) stability to oxidising agents; and (5) reversible reduction to leuco-compounds.

The concentration of these lyochromes in their natural sources is very low. Fresh liver or kidney contains 10–20 mg. per kg., whey contains up to 80 mg. per litre, consisting of a mixture of related lactoflavins, *a*—*d*.

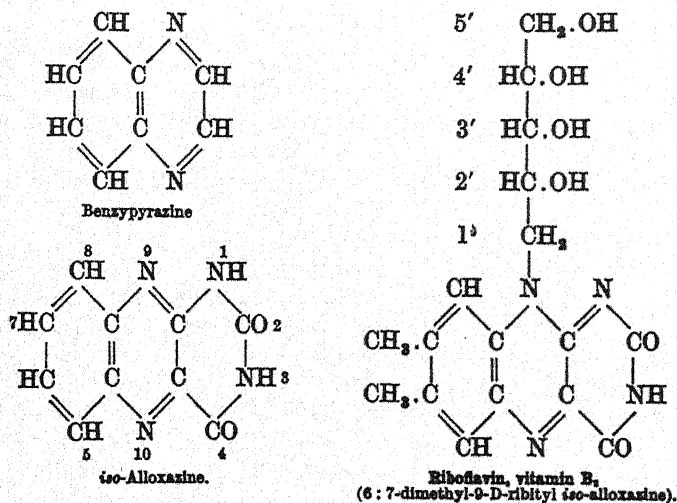
Riboflavin, lactoflavin or vitamin B_2 , $C_{17}H_{20}O_6N_4$, the most important of the lyochromes, occurs in free and bound forms.

(1) *Riboflavin-5'-phosphoric acid*, or *riboflavin mononucleotide*, was isolated from heart muscle, in 1932, by Banga and Szent-Györgyi, and later shown to be the coloured prosthetic group in Warburg's original "yellow enzyme." In riboflavin mononucleotide, the $-O-PO(OH)_2$ group is attached to the terminal or 5' position in the ribityl side-chain.

(2) *Riboflavin-adenine dinucleotide* is the necessary prosthetic group in several flavoproteins that have catalytic properties.

(3) *Free Flavins*.—Principally riboflavin, which is identical with the ovoflavin, hepatoflavin, and lactoflavin *d*.

Structure of the Flavins.—When riboflavin is irradiated in alcohol, a sugar, D-ribose, is split off, leaving a crystallisable residue, *lumichrome*. Alkaline hydrolysis of lumichrome yields urea and a dimethyl benzpyrazine, which suggests the presence of three rings in the original flavin, one of which has yielded the urea. This has led to the *iso*-alloxazine formula now adopted for the lyochromes, according to which lumichrome is the 6 : 7-dimethyl derivative.



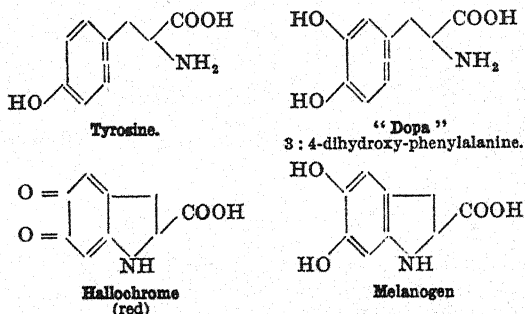
MELANINS

Melanin Pigments.—Tyrosine is oxidised by the enzyme tyrosinase to a red indole compound formed by closure of the side-chain. This red compound is reduced spontaneously to an indole base, *melanogen*, which subsequent oxidation polymerises to a dark-brown pigment, *melanin*. Melanin, or closely related

melanoids, impart the characteristic colour to brown or black hair, the fur of animals, the choroid coat of the eye, the ink secretion of the cuttle fish *Sepia*, and the malignant melanotic sarcoma. The pigments are very stable, and are insoluble in water, acids, and organic solvents, but bleached by oxidising agents, including hydrogen peroxide. The changes involved in melanin formation have been elucidated by Raper (1928).

This series of reactions is of importance in establishing a relationship between tryptophane and tyrosine. The colourless melanogen is excreted in the urine of patients suffering from melanotic sarcoma, and may be recognised by its oxidation to melanin, which occurs spontaneously on exposure to the atmosphere, or after addition of any mild oxidising agents. A corresponding melanin is obtained from the plant amino acid, 3:4-dihydroxy-phenylalanine, by the specific enzyme, "dopa" oxidase, which has no action on tyrosine. The condition of albinism is ascribed to the lack of one or more of these oxidising enzymes.

Melanin formation in the deeper layers of the epidermis is responsible for the racial and climatic pigmentation of the skin, and may have some protective effect against excess of harmful solar radiation.



Hallochrome, first got from the annelid worm, *Halla parthenopea*, seems to be identical with the pigment of red hair, which can be extracted by boiling with N/10 HCl.

PTERINS

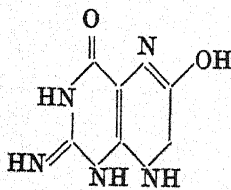
In 1889, Hopkins showed that the yellow pigment of butterfly wings is a cyclic nitrogen compound. Forty years later, the observation was confirmed and extended by Wieland, and by Hopkins, and it was established that these pterins, or wing-pigments, including one from the yellow bands of the wasp, are derivatives of a base, **xanthopterin**, which is formed by union of a pyrimidine and a pyrazine ring. While pterins are almost entirely restricted to insects,

traces occur in mammalian liver and urine, and there is evidence that the anti-anæmic factor in gastric extracts is a pterin derivative (Jacobson and Subbarow, 1937).

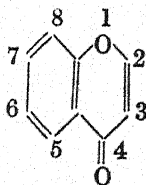
FLAVONES AND ANTHOCYANINS

The widely distributed yellow or orange pigments of flower petals and some fruits are glycosides of a phenyl derivative of chromone, termed **flavone**, or *anthoxanthin*. These flavones are entirely different in structure and properties from the *flavin* pigments; but like them, are of interest in animal physiology, since the anti-sterility vitamins E are chromone derivatives, and the capillary-maintenance vitamin P is closely associated with the flavone fraction of citrus peel, and may be a flavone derivative.

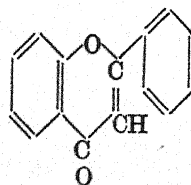
The familiar red, purple or blue colours of flowers, fruits and some leaves are due to glycoside pigments formed from anthocyanins, also indirectly derived from chromone.



Xanthopterin.
(a pterin)



Chromone.
(Benz-1:4-pyrone).



Flavone.
(2-phenyl chromone).

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CHAPTER 13

CATALYSTS

"Catalysis is one of the most significant devices of nature, since it has endowed living systems with their fundamental character as transformers of energy, and all evidence suggests that it must have played an indispensable part in the living universe from the earliest stages of evolution."

F. G. HOPKINS.

ORGANIC life is manifest in a continuum of chemical reactions, the course and rate of which are determined by the presence of various catalysts. These agents usually reveal themselves by activating or rendering unstable certain compounds or substrates, and thereby bringing about chemical changes.

Chemical Reactions.—These involve the redistribution of atoms and the reconstruction of molecules. They exhibit (i.) *direction*, (ii.) *rate*, and (iii.) *extent*.

The *direction* of a reaction is expressed by the change :—

Reactants \longrightarrow End-products.

The *rate* of a reaction is defined as the quantity of substance transformed (removed or produced) in unit time.

The *extent* of a reaction is measured by the percentage change in the concentrations of the constituents.

While the three properties of direction, rate, and extent are dependent primarily on the chemical nature of the reactants, they are liable to be modified in various ways, of which the following are the most important :—

- (1) *Concentration of Reactants.*—At constant temperature, the rate of a reaction at a given instant is proportional to the product of the concentrations of the reactants at that instant.

$$\text{Rate} \propto [a] [b] \dots [z],$$

where [a], [b], [z] denote the *concentration*, or, more exactly, the *activity*, of each reactant (Law of Mass Action).

- (2) *Concentration of End-products.*—Reactions are retarded both by decrease in reactants and increase in end-products,

until finally a state of equilibrium is reached when the composition of the mixture remains constant.

- (3) *Temperature*.—The rate of a simple chemical reaction is increased approximately two-fold for each rise in temperature of 10°C ., within critical limits.
- (4) *Catalysis*.—Many reactions are highly susceptible to the influence of specific agents, or catalysts, which cause an enormous increase in the reaction rate without being used up in the process. Negative catalysts, or reaction inhibitors, are also known.

Catalysed reactions are characterised by :—

- (1) *Survival of the Catalyst*.—Unlike other reactants it is not necessarily removed by the reaction.
- (2) *Continuity of Effect*.—A minimal amount of the catalyst is able to affect the transformation of an indefinitely large amount of reactant.

For example, a highly active sucrase has been prepared that can hydrolyse ten times its weight of sucrose per second for an indefinite time, provided the conditions are kept favourable.

- (3) *Independence of Equilibrium*.—The final state of a chemical reaction is independent of the way in which the state has been reached. Catalysts accelerate the speed at which equilibrium is attained, without altering significantly the final composition of the equilibrium system.

Definitions of a Catalyst.—(1) "A catalyst is a substance that accelerates a reaction but cannot induce a reaction" (Bodenstein, 1902).

(2) "In a catalysed reaction the chemical composition of one reactant is the same as that of one of the products—this substance is the catalyst" (Falk, 1922; Northrop, 1926).

The definition of a catalyst as a reaction-accelerator when first proposed by Ostwald stimulated research into the kinetics of catalysed reactions. Falk's definition is less ambitious and more precise, and furthermore avoids the objection that many reactions susceptible of catalysis do not appear to take place at all in the absence of the catalyst.

Examples are the stability of sucrose and of urea at ordinary temperatures, provided the solutions be kept sterile, a precaution generally overlooked by earlier workers.

(3) "A catalyst is a substance which enables certain molecules to undergo chemical changes on receiving a critical energy that is less than they would require in the absence of the catalyst" (Moelwyn-Hughes, 1933).

Biological Catalysts.—The catalysts manufactured and used by living organisms appear in two forms : (a) *catalytic surfaces*, such as occur inside cells ; (b) *catalytic solutes*, such as are found dissolved in secretions and tissue extracts. The distinction between the two forms is not final ; by disintegration, catalytic surfaces may be removed as solutes, and, conversely, inside the cell many catalytic solutes may be concentrated in particular regions.

Catalytic solutes may be subdivided into (i.) *non-colloidal* catalysts, and (ii.) *colloidal catalysts* or enzymes. The first subgroup includes soluble catalysts of low molecular weight, such as electrolytes, glutathione and ascorbic acid ; the second group includes the vast assortment of enzymes that in variety and importance dominate the biochemistry of catalysis.

(i.) *Non-colloidal Catalysts*

H-ions.—Catalyse the hydrolysis of disaccharides and esters, and many other organic reactions.

HO-ions.—Catalyse the oxidation of polyphenols, the isomeric transformation of monosaccharides, the conversion of atropine into hyoscyamine, and other changes.

Cu-ions.—Catalyse the dehydrogenation of ascorbic acid, and many other oxidations.

(ii.) *Colloidal Catalysts*

Metallic Sols.—Metals in colloidal form modify many chemical reactions, usually those concerned with oxidation or hydrogenation, as, for example, the use of nickel in the hardening of oils into fats.

Enzymes.—Biological catalysts obtainable in soluble, colloidal forms.

Biological, because they are produced by living organisms.

Soluble, because they occur in secretions or can be extracted from tissues by means of aqueous solvents.

Colloidal, because the molecular dimensions are greater than $1\text{ m}\mu$, and the particles in consequence will not pass through collodion or parchment membranes.

Enzymes are defined by Waldschmidt-Leitz (1936) as : Catalysts of a definite organic nature with a specific activity, formed only by living cells but acting independently of living cells.

(iii.) *Insoluble Catalysts*

Catalytic Surfaces.—These may be specialised areas of the cell-wall mosaic or may be enzymes immobilised in various ways on cell-inclusions. Willstätter (1933) has shown that in addition

to the soluble, or *lyo*-enzymes, a class of bound or *desmo*-enzymes exists which can only be separated after the parent tissues have undergone autolysis (self-digestion), or have been extracted with organic solvents.

The History of Enzyme Chemistry.—Four spontaneous natural changes have been known to man ever since he first acquired the power of rational observation. They are : (i.) the alcoholic fermentation of sugars ; (ii.) the lactic fermentation, or souring, of milk ; (iii.) the acetic fermentation of wine ; and (iv.) the ammoniacal fermentation of urine. Each is due to the growth of a microscopic organism and its attack on a particular substance or *substrate*. The mechanism of these natural fermentations remained completely obscure until the beginning of the last century.

(1) In 1830, Dubrunfaut found that malt extract could convert starch paste into sugar in a manner comparable to the action of a strong acid, as shown previously by Kirchoff (1815). In 1833, Payen and Persoz separated the active amylolytic principle from malt extract by addition of excess of alcohol. This precipitate, the first true enzyme isolated in the crude state, they named *diastase*, and compared it to one of the unknown natural "ferments" that brought about the souring of milk or wine. The wide distribution and importance of these ferments was realised, and Berzelius introduced the term *catalysis* to describe the changes that they brought about.

(2) Between 1850 and 1870, Pasteur showed that the natural fermentations were invariably due to the growth of micro-organisms, called by him the "organised ferments." Non-living agents, such as the pepsin of gastric juice or the diastase of malt extract were called the "soluble or unorganised ferments."

(3) Confusion having arisen as to the meaning of "ferment," Kühne, in 1870, introduced the term *enzyme* to describe biological catalysts irrespective of their origin.

(4) In 1896, Ostwald defined a catalyst as the accelerator of a chemical reaction. This stimulated research into the physical chemistry of enzymes, and many equations were obtained connecting speed of zymolysis with concentration of catalyst, substrate, and end-products.

(5) Since 1900, enzyme research has developed to such an extent that each single enzyme or a single property of a class of enzymes has proved sufficient to engage the attention of a group of investigators. Important dates are : 1926, crystallisation of urease (Sumner) ; 1932, discovery of flavin enzymes (Warburg) ; 1933, isolation of the co-enzymes ; 1935, isolation of virus protein ; 1940, synthesis of starch and glycogen.

Properties of Enzymes.—In addition to the general endowments of all catalysts, namely, (i.) power of survival, (ii.) continuity of effect, and (iii.) independence of equilibrium, the enzymes display special properties of their own, including :—

- (1) Colloidal characteristics.
- (2) High selectivity.
- (3) Sensitivity to temperature.
- (4) Sensitivity to H-ion concentration.
- (5) Sensitivity to electrolytes.
- (6) Sensitivity to specific factors, including co-enzymes, activators and toxins.

A particular enzyme can only catalyse one type of reaction or one class of related reactions, and thus differs from most inorganic catalysts, which are usually less discriminating.

Colloidal Characteristics.—In secretions and extracts, enzymes are closely accompanied by inert colloids, usually proteins, the removal of which is the greatest problem in purification. However, even when in the highest state of purity yet attained, enzymes themselves are colloidal solutes of low diffusibility and high molecular weight.

The colloidal state is determined by the size of the solute particle in reference to the solvent. For aqueous solvents, the range is $1\text{ m}\mu$ – $100\text{ m}\mu$, above which the particles are too large to form stable dispersions. The size of the particles may be found by various methods, including: (i.) ultra-filtration through membranes of known porosity, (ii.) rapidity of diffusion, and (iii.) fractionation by centrifuge accompanied by observation of diffusion rate.

Approximate Molecular Weight and Diameter of Typical Enzymes

Enzyme.	Molecular Weight.	Diameter in $\text{m}\mu$.
Pepsin	35,000 or 70,000	4.2
Trypsin	34,000–45,000	4.4–5
Emulsin	37,700	4.2
Urease	480,000	—
Catalase	250,000	—

These data show that some enzymes are of the same order of magnitude as the heat-coagulable proteins.

Selectivity.—By this is meant the restriction of the catalytic effect to one particular substrate or a group of substrates. Three types of selectivity are recognised :—

- (a) *Absolute specificity of attack*, when the enzyme acts only on one substrate, or one type of linkage in a class of substrates. The decomposition of urea by *urease* is an example.
- (b) *Relative specificity of attack*, when an enzyme acts on two or more substrates, usually with different intensities. The hydrolysis of esters by *lipase* is an example.
- (c) *Specificity of reaction path*, when an enzyme decomposes a substrate in a particular way, although the final products may be the same as those obtained by the aid of other catalysts. Thus, when the trisaccharide raffinose is hydrolysed by yeast *saccharase*, it yields fructose and melibiose. When hydrolysed by *emulsin* it liberates sucrose and galactose.

Temperature Sensitivity.—This is shown by two important characteristics—heat-inactivation and heat-acceleration. The irreversible inactivation by heat is used to distinguish enzymes from non-enzymatic catalysts, and is one of the means of detecting enzymes.

All known enzymes are inactivated, or "killed," by being boiled in aqueous solution.

Sensitivity to temperature is increased by purification and by certain conditions of H-ion and electrolyte concentrations, hence the temperature of "thermal inactivation" is not sufficiently precise to be used for identifying a particular enzyme.

Heat-inactivation is ascribed to irreversible changes in the colloid enzyme comparable with the heat-coagulation of higher proteins.

Heat-acceleration is shown by the existence of an optimal temperature range for every reaction, although the limits of the range are modified by various factors, including the gradual inactivation of the enzyme by prolonged heating.

Optimal H-ion Values for Typical Enzymes

Enzyme.	Source.	Substrate.	Optimal pH.
Pepsin	gastric mucosa	proteins	1.5-2.5
Rennin	" "	caseinogen	6-6.5
Peroxidase	plant roots	hydrogen peroxide	3
Phosphatase	<i>Aspergillus</i>	glycerophosphate	3-6
Zymase	yeast	glucose	4.5-6.5
Lipase	gastric mucosa	fats	5.5-8.6
Amylase	malt	starch	5.2
Amylase	pancreas	starch	6-7
Urease	Soy bean	urea	7-8
Trypsin	pancreas	proteins	8-9
Phosphatase	cartilage	pyrophosphates	7.5
Phosphatase	cartilage	phosphoric esters	9.0-9.2

Sensitivity to H-ion Concentration.—All enzymes display a region of optimal H-ion concentration, which varies from about pH 1.5 for pepsins up to about pH 10.0 for trypsin and mammalian phosphatase. The majority act best within the range pH 4.5–pH 7.5.

As well as optimal pH values there are maximal and minimal limits beyond which the enzyme does not act. Exposure to these extremes is often marked by destruction of the catalyst in a manner comparable with thermal inactivation.

Sensitivity to Activators.—Many enzymes are powerfully affected by specific ions or simple solutes, which either accelerate or inhibit the reaction. Activators are divided provisionally into *promoters*, that increase the rate at which the reaction starts; and *protectors*, that prolong the rate by retarding the spontaneous inactivation of the enzyme.

Promoters.—In low concentration, Mg ions activate phosphatase; Ca ions activate pancreatic lipase; Co^{++} and Mn^{++} activate arginase; Cl ions probably are necessary for amylase; Fe^{+++} participates in various oxidation systems.

Cyanide, hydrogen sulphide and other thiol (—SH) compounds, including cysteine and reduced glutathione, in low concentration, activate papain, cathepsin, urease and other enzymes.

Protectors.—Agents capable of neutralising toxic contaminants or by-products during zymolysis. Thus, α -amino acids protect urease, arginase and probably other enzymes.

Co-enzymes.—Heat-stable organic compounds naturally associated with complex enzyme systems, and necessary for zymolysis. They act as carriers of hydrogen, phosphate or other intermediate reactants in the system.

Co-dehydrogenase I, co-enzyme I or co-zymase, obtainable from extracts of yeast, muscle, liver and kidney, is the co-enzyme of sugar fermentation, and acts with the dehydrogenases of hexose diphosphate, malate, and alcohol, and also with the lactate dehydrogenase of muscle.

Co-dehydrogenase II, co-enzyme II (Warburg), obtained from yeast and red blood cells, acts with hexose monophosphate dehydrogenase.

Riboflavin-mononucleotide and *riboflavin-adenine-dinucleotide*, co-enzymes of the *flavin-enzyme* systems.

Co-carboxylase, the pyrophosphoric ester of vitamin B_1 .

Glutathione acts as a co-enzyme for glyoxalase and for the intracellular proteinases of animal tissues.

Inhibitors and Inactivators.—These include ions of heavy metals, especially silver and mercury, aldehydes and nitrous acid, which attack amino groups; amino compounds, which combine with aldehyde groups; relatively weak oxidising agents; reducing agents in high concentration, and the more specific *anti-enzymes* found in blood and tissues. Inactivation is reversible or irreversible, and may be *competitive* (p. 256).

CLASSIFICATION OF ENZYMES

Enzymes are classified according to the compound, or **substrate**, they act upon, the name of the enzyme or enzyme type being

formed by attaching the suffix *ase* to the name of the substrate. Thus, *esterases* activate esters, and *urease* decomposes urea. The older names, such as pepsin and trypsin, are still retained, partly because of long established usage, and partly because some of them apply to groups of closely related catalysts.

Many enzymes are conveniently classified in terms of their function rather than their substrates.

A. HYDROLASES

Enzymes causing hydrolytic decomposition of the substrate by attacking, or *activating*, specific linkages.

(1) **Esterases.**—Attack the ester linkage, $-\text{CO.O}-\text{CH}=\text{}$ or $\text{A}-\text{O}-\text{CH}=\text{}$, where *A* is an acid radicle. *Phosphatases* form an important class of *esterase*, and activate the linkage $\text{R}-\text{O}-\text{PO}(\text{OH})_2$, liberating phosphoric acid.

(2) **Carbohydrases.**—Convert higher carbohydrates into simple sugars.

(a) **Polysaccharidases** (Polyases).—Depolymerise polysaccharides.

(b) **Saccharidases** (Hexosidases and Pentosidases).—Attack the oxygen linkage, $-\text{CH}(\text{OH})-\text{O}-\text{CH}=\text{}$, in compound saccharides.

Proteoclastic, or proteolytic enzymes include :—

(3) **Proteases.**—Attack the peptide linkage, $-\text{CO}-\text{NH}-$, in proteins.

(a) **Proteinases.**—Preferentially attack central peptide linkages.

(b) **Peptidases.**—Only attack terminal peptide linkages.

(4) **Aminases.**—Attack the amino group or the imino linkage, $=\text{CH}-\text{NH}-$, in amino and related compounds.

B. OXIDO-REDUCTASES

Enzymes activating oxidation-reduction systems, one component of which is the substrate.

(1) **Dehydrogenases** (Dehydrases).—Transfer hydrogen from the substrate to a hydrogen acceptor, thus effecting an indirect oxidation.

(2) **Oxidases.**—Activate the oxygen linkage, $-\text{O}-\text{O}-$, in molecular oxygen, peroxides and other oxygen donors.

C. DESMOLASES

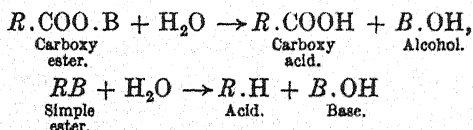
Enzymes causing resolution of the $-\text{C}-\text{C}-$ bond.

1. ESTERASES

Representative Esterases

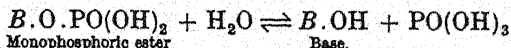
Name.	Source.	Substrate.	End-products.
Pancreatic lipase .	pancreas	fats and other	aliphatic acids and
Gastric lipase .	stomach	organic esters	glycerol.
Plant lipase . .	seeds	"	"
Liver lipase . .	liver	simple " esters"	acid " and alcohol.
Phosphatase . .	yeast	phosphoric esters	phosphoric acid and
	cartilage		base
Sulphatase . . .	fungi	sulphuric esters	acid sulphates and
			phenols.
Tannase	fungi	tannins	gallic acid and glu-
			cose.
Chlorophyllase .	green leaves	chlorophyll	chlorophyllin and
			phytol.
Choline esterase .	nerve	acetyl choline	choline and acetate

The esterases hydrolyse carboxylic and other esters in accordance with the equations :—



Lipases.—These esterases preferentially activate fats. The chief are pancreatic lipase and plant lipase. The latter occurs in all seeds rich in oil, its commonest source being the castor oil bean (*Ricinus communis*). Castor lipase is insoluble in water, and is extracted along with the lipide in an oily emulsion.

Phosphatases attack a variety of phosphoric esters, including glycerophosphates, hexosephosphates, and nucleotides. They are widely distributed in yeasts, moulds, and other sugar-fermenting organisms, and in mammalian tissue, chiefly intestinal mucosa, nervous tissue, bone, and ossifying cartilage. They liberate free phosphoric acid in accordance with the reversible reaction :—



The simple phosphatases, or *phospho-monoesterases*, liberate H_3PO_4 and an alcohol from a great variety of organic monophosphates, including α -glycerophosphate, β -glycerophosphate and hexose phosphate, thus releasing reactants of importance in carbohydrate, lipide and phosphate metabolism. Two classes of simple phosphatase exist :—

The "acid type," with an optimum about pH5, occurs chiefly in prostate, liver, pancreas and rice bran.

The "alkaline type," optimum pH9, occurs chiefly in kidney, leucocytes, intestinal mucosa and bone. Mg ions inhibit phosphatases of the "alkaline" but not those of the "acid type". Measurement of the two types in serum is of value in diagnosis of bone disease and malignant changes in the prostate, in which the output of the "acid type" is increased.

W. Jessop has developed an effective method for the histological detection of phosphatase; in which the tissue is incubated with glycerophosphate and lead acetate, which forms a precipitate with the released PO_4 (1944).

Nucleotidase, which appears to be an "alkaline type" phosphatase, occurs in glandular and nerve tissue, blood and the intestinal mucosa. It hydrolyses nucleotides to nucleosides and H_3PO_4 .

Phosphodiesterases hydrolyse one linkage in diphosphates, and thus form monophosphates capable of being attacked by a monoesterase.

Lecithinase, found in kidney and other animal tissues, yeast and snake venom, is a diesterase, and removes the glyceride residue from lecithin.

Phytase, from malt, bran, fungi and liver, hydrolyse phytic acid to inositol and H_3PO_4 .

Pyrophosphatases attack the inter-phosphate linkage, and thus release H_3PO_4 from triphosphoric esters, which are degraded into pyrophosphates, and, by loss of another H_3PO_4 , into phosphates. These enzymes are of importance in controlling the reversible release and uptake of H_3PO_4 in energy-exchange reactions, such as the intermediate metabolism of carbohydrates.

Adenosine triphosphatase, now believed to be identical with *myosin*, the chief protein in muscle, resolves its substrate into *adenosine pyrophosphate* and H_3PO_4 , thereby initiating muscular contraction.

The enzyme is retarded by slight increase in temperature ($>40^\circ$) or acidity ($<\text{pH } 4$), and is inactivated by Ag ions.

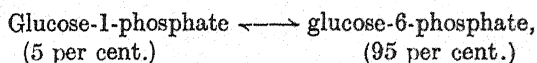
Thiamine pyrophosphatase converts its substrate, *Co-carboxylase*, into *thiamine phosphate* (vitamin B_1 phosphate). It is widely distributed along with the vitamin. The optimal pH for the yeast enzyme is 3.7.

Phosphorylases catalyse the reversible conversion of inorganic phosphate and starch or glycogen into glucose-1-phosphate. Using phosphorylase, from potato, Hanes (1940) has been able to synthesise a starch from pure glucose-1-phosphate.

A similar glycogen-phosphorylase occurs in yeast, liver and brain, and has been isolated in crystalline form from muscle by Green, Cori and Cori (1942). At pH7, it catalyses the equilibrium :—



Phospho-glucomutase, from yeast and animal tissues, catalyses the mutation equilibrium :



a transformation necessary both in the degradation and assembling of sugars (Colowich and Sutherland, 1942).

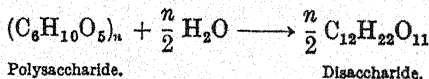
2A. POLYSACCHARIDASES

Polysaccharidases attack polymerised carbohydrates, such as starch and cellulose, and convert them into sugars.

Polysaccharidases

Name.	Source.	Substrate.	End-products.
Amylase (diastase) {	pancreas	starch,	maltose.
Cellulase . . .	malt	glycogen	
Inulase . . .	fungi	cellulose	cellobiose.
	plants	inulin	fructose.

Amylase, or diastase, denotes a family of enzymes characterised by their power of depolymerising starch (amylum) and glycogen, with the production of a series of intermediate dextrins, leading eventually to maltose.



Amylases are accompanied often by maltases, and, as a result, the end-product of the zymolysis is glucose, formed by the action of the maltase on the disaccharide. Amylases occur in the liver and digestive secretions of animals, in the roots, seeds, and germinating shoots of plants, and in many micro-organisms. The saliva of man the pig, the rat, and other mammals contains an amylase termed **ptyalin** ; it is absent usually from the saliva of dogs and carnivora. The pancreas is the richest source of the enzyme in the animal. Industrially, amylase is obtained in quantity from extract of malt, or germinating barley.

Malt amylase has been resolved into a dextrinogen, α -amylase, which attacks central glucoside linkages in the starch molecule; and β -amylase which splits off maltose units step by step from the ends of the parent molecule.

The amylolytic hydrolysis of starch is explicable by the laminated formula (p. 106), assuming that the cross-links uniting the long-chains are resistant to β -amylase, but are broken by α -amylase from malt or saliva (Haworth *et al.*, 1943).

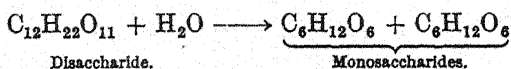
Cellulase is secreted by many bacteria and soil organisms, by wood-destroying fungi, and some marine worms. It is a necessary agent for all organisms that attack the natural cellulose of wood, leaf-mould, and timber. Cellulose is broken down into the disaccharide cellose (cellobiose), which, in turn, is converted into glucose by the enzyme *cellobiase* that often accompanies cellulase.

Cellulase is not found in higher animals; the celluloclastic powers of herbivora are ascribed to the presence of micro-organisms in their digestive tracts.

2B. SACCHARIDASES

Saccharidases attack compound sugars and convert them into simple sugars.

Three *trisaccharidases* are known (raffinase, melizitase, and stachyase), the rest of the group being *disaccharidases*, or enzymes hydrolysing disaccharides in accordance with the simple equation:—



Disaccharidases

Name.	Source.	Substrate.	End-products.
Maltase . . .	malt, intestinal secretions	maltose	glucose + glucose.
Sucrase, or saccharase (invertase).	yeast, intestinal secretions	sucrose	fructose + glucose.
Emulsin . . .	plants	glucosides	aglucone + glucose.
Lactase . . .	intestinal secretions	lactose	galactose + glucose.
Cellobiase . . .	<i>Aspergillus</i> fungi	cellobiose	glucose + glucose.

Maltase accompanies amylase in animal tissues and completes the hydrolysis of polysaccharides. It is present in many bacteria, fungi, and yeasts, and in an insoluble form in ungerminated cereals. The optimal range of activity is about pH 6.1 (intestinal maltase) to pH 4.1 (malt-extract maltase).

Sucrase, saccharase or invertase is very widely distributed, occurring in the intestinal secretions of most animals, including the bee (as honey invertase) and the snail. It is found in all parts of plants, sugar beet being particularly rich, in fungi, bacteria, and yeasts (except true wine yeasts). All these preparations of the enzyme hydrolyse or "invert" cane sugar (sucrose) into "invert sugar," an equimolecular mixture of fruit and grape sugar.

Lactase occurs in the intestine of all young mammals, although it may disappear in later life unless milk be part of the diet. It occurs in plants, especially almond seeds, *Aspergillus oryzae*, and the milk-sugar yeasts, but not in "top-fermentation" yeasts. Lactase converts lactose into galactose and glucose at the optimal pH of 4.3 for almond lactase, and 7.0 for yeast lactase.

3A. PROTEINASES

Proteoclastic enzymes can be divided into two groups, the proteinases or *endopeptidases* which are capable of attacking central in preference to terminal peptide linkages; and *exopeptidases* (formerly termed peptidases or ereptases), which only attack terminal linkages. All enzymes which attack native proteins, albumins, globulins and their higher derivatives, split off many different polypeptides from the large parent molecule, and for this reason are classified as endopeptidases. Both groups of enzymes are necessary for the complete hydrolysis of proteins.

Three chief types of proteinase occur: *Pepsinases*, acting in acid solution; *trypsinases*, acting in alkaline solution; and *papainases*, acting on proteins in neutral solution.

Representative Proteinases (Endopeptidases)

Name.	Source.	Substrate.	End-products.
Pepsin *	stomach	proteins	peptones.
Trypsin	pancreas	tissue "	"
Cathepsin	spleen, etc.	tissue protein	"
Papain	melon tree	native protein	peptides.
Bromelin	pineapple	"	"
Rennin	stomach	caseinogen	para casein.
Protaminase	intestine	protamines	peptides.

Pepsin.—The digestive power of gastric juice on meat protein was demonstrated by Spallanzani, about 1780, and the agent was located and named "pepsin" by Schwann in 1836. It is the only mammalian enzyme that acts in a strongly acid medium equal to N/10 HCl. At a pH less acid than 4, the activity of pepsin rapidly

approaches zero. Pepsin attacks all native proteins except the protamines and the scleroproteins.

Pepsin is secreted as an inactive *pepsinogen*, which is autocatalytically changed to pepsin in acid solutions.

Trypsin.—The protease of pancreatic juice is secreted in an inactive form termed **trypsinogen**, and is activated by the enzyme enterokinase of the intestinal secretion.

Trypsin resembles pepsin in its power of attacking native proteins. In the pancreatic juice it is accompanied by various peptidases which act on the peptones produced by trypsin (and pepsin), and convert them into amino acids. The presence of these peptidases as contaminants was only established in 1929, it being formerly believed that trypsin could exert a peptoclastic property of its own.

Trypsin differs sharply from pepsin in its pH range. It works only in alkaline solutions of about pH 8–pH 11. The work of Bergmann shows, however, that the pH range of pepsin, papain and trypsin depends largely on the type of substrate attacked (1938).

Isolation of the Proteinases.—Working on the assumption that enzymes are protein in structure, Northrop and his associates (1930–39) applied routine methods of protein fractionation by MgSO_4 and $(\text{NH}_4)_2\text{SO}_4$ to crude pepsin and pancreatic extracts, and thereby isolated both the enzymes and their precursors in crystalline form. The validity of such claims lies in the fact that repeated purification by recrystallisation does not change the catalytic potency of the preparations.

Pepsin crystallises in characteristic hexagonal bi-pyramids; *pepsinogen* appears in fine needles. *Trypsin*, *trypsinogen*, *chymotrypsin* and *chymotrypsinogen*, have also been crystallised. The *zymogens*, pepsinogen, trypsinogen and chymotrypsinogen, are much less heat-labile than the derived enzymes.

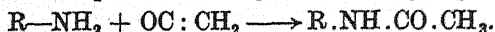
Trypsinogen spontaneously changes to trypsin in alkaline solutions at pH 7–9; in acid solutions at pH 5.2–6, the change is catalysed by *enterokinase*, which was discovered by Pavlov in intestinal juice and pancreas, and shown to be an enzyme by Kunitz (1938–41). Chymotrypsinogen is activated by trypsin but not by enterokinase. An anti trypsin occurs in blood plasma, and also has been isolated as a crystalline polypeptide from pancreas by Northrop and Kunitz.

Distinctions between Pepsin and Trypsin.—According to Hugounenq and Loiseleur (1926), pepsin retains its proteoclastic activity after its free $-\text{NH}_2$ groups have been methylated by formaldehyde or destroyed by nitrous acid. Proteins treated in the same way are no longer attacked by pepsin.

Trypsin, on the other hand, is destroyed by methylation or

deamination, although untreated trypsin is able to attack proteins that have been methylated or deaminated. From this, we conclude that pepsin functions by means of its carboxyl groups interacting with the free amino groups of the protein substrate; while trypsin functions by means of its amino groups interacting with the carboxyl groups of the substrate.

A more exact discrimination is by means of *ketene*, $\text{H}_2\text{C}:\text{CO}$, which, at room temperatures, acetylates amino groups:



By careful acetylation, it can be shown that pepsin still operates when its amino groups are no longer free. Protracted action of ketene inactivates pepsin, probably by acetylation of essential hydroxyl groups on the tyrosine units in the enzyme:



Rennin, Rennet, Chymase.—A phosphoproteinase found chiefly in gastric juice, but also present in the pancreas, and various other animal and plant tissues.

In the presence of calcium it converts soluble caseinogen into insoluble casein (paracasein), the optimal pH being 6.0–6.5. This causes the coagulation of milk.

Cathepsin.—The chief representatives of intracellular proteinases found in animal tissues, and responsible for autolytic digestion. Cathepsin of the papainase type attack proteins in neutral solution, and is activated by thiol compounds (reduced glutathione, hydrogen sulphide) and by cyanide. Cathepsins I (pepsin type), II (trypsin type), and III (aminopeptidase type) occur in spleen and kidney.

Distinction between Pepsin and Rennin.—Pepsin, even from pure, recrystallised preparations, can coagulate milk in a manner similar to rennin. It differs in its optimal range pH 1.5–1.7, and it can attack a great variety of proteins, whereas rennin is species-specific for caseinogen. A separation of the two enzymes by fractional precipitation of rennin by alcohol at pH 5.4 has been described (Tauber, 1937). At the pH of normal gastric contents, rennin is inactive.

3B. PEPTIDASES (EXOPEPTIDASES)

These widely distributed enzymes differ from pepsin and purified trypsin by the fact that though unable to attack native proteins they act readily on peptides of all degrees of complexity, and resolve them into amino acids. **Erepsin** is an important mixture of peptidases found in the secretion of the intestinal mucosa. Similar mixtures are obtained from higher plants, yeasts, and micro-organisms. They can be resolved into peptidases displaying a high

degree of specificity for different peptides. In order to be susceptible to peptidase attack the peptide must contain at least one free amino or one free carboxyl group. *Dipeptidases* require both groups in their substrate.

Natural digestive secretion, such as gastric and pancreatic juice, gland and tissue extracts and unicellular organisms contain characteristic mixtures of proteases whereby they are able to resolve a variety of proteins into the constituent amino acids.

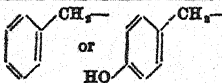
Proteoclastic Systems of Different Origin (Waldschmidt-Leitz, 1936)

Pancreas-intestine.	Glandular Tissue.	Yeast.	Leucocytes.
Pancreatic-proteinase (trypsin-type) Protaminase Amino-peptidase Carboxypeptidase	Cathepsin (papain-type) Amino-peptidase Carboxypeptidase Dipeptidase Prolinase	Proteinase (papain-type) Amino-peptidase Carboxypeptidase Dipeptidase Prolinase	Proteinase (trypsin-type) Cathepsin Amino-peptidase Carboxypeptidase Dipeptidase

Classification of Proteinases (Bergmann, 1942).—Proteoclastic, or proteolytic enzymes hydrolyse peptide-bonds, the process being reversible. Each enzyme requires special groupings in the main chain of its protein substrate, and also special groupings in the side-chains. Thus, a *carboxypeptidase* removes units containing free $-\text{COOH}$, while *amino-peptidase* attacks units containing free $-\text{NH}_2$, *endopeptidases*, such as pepsin and trypsin, require, in proximity to their point of action, a second peptide bond.

Classification of Proteinases (Bergmann, 1942)

I. Endopeptidases

Enzyme.	Reaction.	Necessary Side-groups, R.
Pepsin, Cathepsin I	$\begin{array}{c} -\text{CO.NH.CH(R).CO.NH.CH(R)-} \\ \downarrow \\ -\text{CO.NH.CH(R).COOH} + \text{H}_2\text{N.CH(R)-} \end{array}$	
Trypsin, Cathepsin II	$\begin{array}{c} -\text{CO.NH.CH(R).CO.NH-} \\ \downarrow \\ -\text{CO.NH.CH(R).COOH} + \text{H}_2\text{N-} \end{array}$	$\begin{array}{c} \text{H}_2\text{N.CH}_2.\text{CH}_2.\text{CH}_2.\text{CH}_2- \\ \text{or} \\ \text{H}_2\text{N} \diagdown \text{CH.NH.CH}_2.\text{CH}_2.\text{CH}_2- \\ \text{H}_2\text{N} \diagup \end{array}$
Chymotrypsin	$\begin{array}{c} -\text{CO.NH.CH(R).CO.NH-} \\ \downarrow \\ -\text{CO.NH.CH(R).COOH} + \text{H}_2\text{N-} \end{array}$	The same as for pepsin.

II. Exopeptidases

Enzyme.	Reaction.	Necessary side-groups, R.
Aminopeptidases, Cathepsin III	$\begin{array}{c} \text{H}_2\text{N} \cdot \text{CH(R)} \cdot \text{CO}-\text{NH}- \\ \downarrow \\ \text{H}_2\text{N} \cdot \text{CH(R)} \cdot \text{COOH} + \text{H}_2\text{N}- \end{array}$	$\begin{array}{c} \text{CH}_2 \\ \diagup \quad \diagdown \\ \text{CH} \cdot \text{CH}_2- \end{array}$
Carboxypepti- dases, Cathepsin IV	$\begin{array}{c} \text{HOOC} \cdot \text{CH(R)} \cdot \text{NH}-\text{CO}- \\ \downarrow \\ \text{HOOC} \cdot \text{CH(R)} \cdot \text{NH}_2 + \text{HOOC}- \end{array}$	The same as for pepsin.

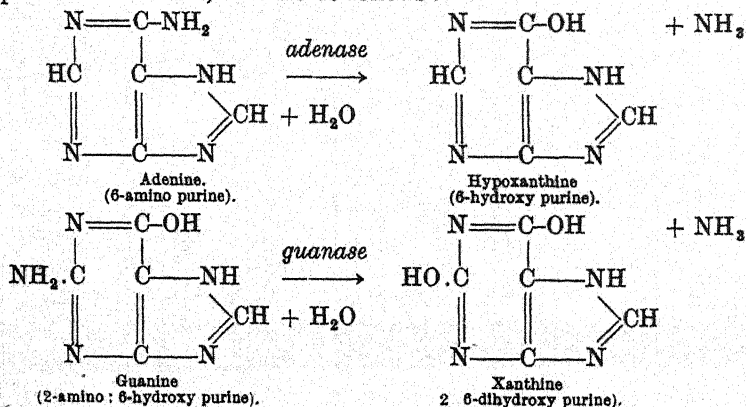
4. AMINASES AND AMINE OXIDASES

A miscellaneous group of enzymes that are capable of activating the linkage $-\text{C}-\text{NH}-$ in non-protein compounds, nitrogen being liberated usually in the form of ammonia. Many of these enzymes are really members of the dehydrogenase family.

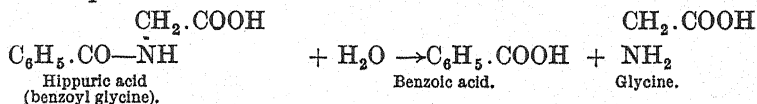
Representative Aminases

Name.	Source.	Substrate.	End-products.
Adenase .	liver	adenine	hypoxanthine.
Guanase .	liver	guanine	xanthine.
Allantoinase .	seeds	allantoin	allantoic acid.
Hippuricase .	kidney	hippuric acid	benzoic acid and glycine.
Arginase .	liver	arginine	ornithine and urea.
Urease .	Soy bean	urea	ammonia and carbon dioxide.
Asparaginase .	Aspergillus	asparagine	aspartic acid.
α -deaminase .	kidney	α -amino acids	keto acids
Glutaminase .	brain	glutamine	glutamic acid.

Adenase and **Guanase** deaminate the purine derivatives, *adenine* and *guanine*, respectively. Both enzymes are found in the liver and spleen of cattle and other mammals. Pig spleen is exceptional in containing adenase only. The changes are important stages in purine metabolism, and are as follows:—

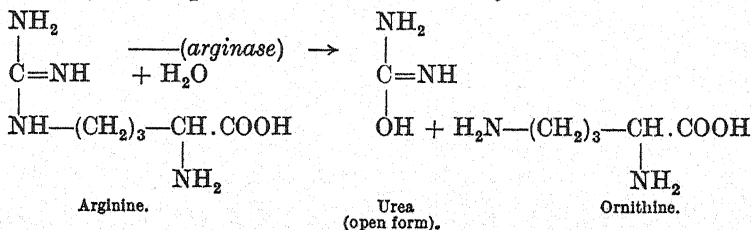


Hippuricase, or histozyme, acts on hippuric acid and its homologues. The enzyme occurs in the liver and also in the kidney, where it may bring about a reversion synthesis of hippuric acid from its components.

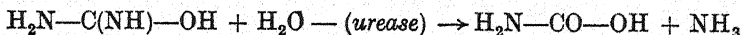


Arginase occurs in the livers of all animals that are *ureotelic* in that they excrete their waste nitrogen chiefly in the form of urea. It is absent from the livers of *uricotelic* animals (birds and most reptiles), which excrete nitrogen as uric acid. Traces of the enzyme may occur in non-hepatic tissue, and it has also been identified in plants.

Arginase specifically hydrolyses natural or (+)-arginine into the diamino acid ornithine and urea, and is part of the mechanism whereby urea is produced in the animal body.

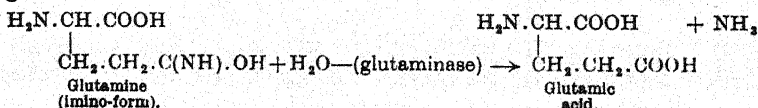


Urease, the enzyme responsible for the alkaline fermentation of urine, is widely distributed through the vegetable kingdom, especially in root nodules and seeds of *Leguminosae*, important sources being the Soy bean, the Sword bean and the Jack bean, from the last of which urease has been isolated as a crystalline protein by Sumner. The enzyme, as might be expected, is absent from the tissues of higher animals, apart from an occasional appearance in gastric juice. Urease rapidly attacks urea, with formation of ammonia and carbamic acid, which breaks down into ammonia and carbon dioxide.



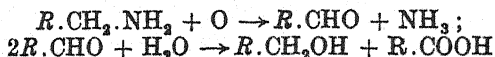
Urease is a specific enzyme, and its use provides an exact method for the detection and estimation of urea in blood and tissues.

Glutaminase occurs in brain cortex and retina of vertebrates, and in rabbit and guinea-pig kidney. It converts glutamine into glutamic acid and ammonia.



AMINE OXIDASES

Biological deamination is usually accompanied by oxidation of the substrate, with the formation of an aldehyde, two molecules of which subsequently react to form alcohol and acid in accordance with the equations :

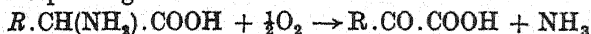


The first reaction is catalysed by the amine oxidase, the second reaction may take place spontaneously or on activation by a separate enzyme, *aldehyde mutase*.

Many of the enzymes termed aminases are really amine oxidases, and confusion may be caused by the overlapping of the terminology, which is aggravated by the fact that amine oxidases are also included among the oxido-reductases in some classification.

Amine oxidase is widely distributed among vertebrate and invertebrate tissues, and attacks many aliphatic and aromatic amines, including adrenaline and tyramine. It is distinct from *histamine oxidase*, which oxidises diamines.

Amino acid oxidase, α -deaminase, converts α -amino acids into their corresponding keto acids :



The process is an aerobic deamination employing molecular oxygen, as denoted by $\frac{1}{2}O_2$.

The enzyme occurs in mammalian liver and kidney, from which sources Krebs (1935) has separated two types : L-deaminase, which attacks the natural amino acids, and which can be inhibited by octyl alcohol; and D-deaminase, which oxidised the optically isomeric D-amino acids, and is not inhibited by octyl alcohol. Since these D-acids are foreign to animal tissues and not found in food proteins, the significance of their deaminase is obscure, unless it be to protect the animal from evolutionary freaks of metabolism.

OXIDO-REDUCTASES

The oxidation of a substrate is always accompanied by the reduction of another compound, usually termed the oxygen-donor or hydrogen-acceptor. Consequently, the enzymes concerned in oxidation are grouped as oxido-reductases, since they may function either by activating the substrate so that it loses hydrogen, or activating the donor so that it loses oxygen. Oxido-reductases include :

- (1) *Dehydrogenases*, a type of highly specific enzymes represented in all living tissues, which bring about oxidations indirectly by transferring activated hydrogen from the substrate to a suitable acceptor. They differ from most of the other oxido-reductases in being inhibited by narcotics,

such as urethane, but not by cyanide, carbon monoxide or hydrogen sulphide in low concentration. The principal dehydrogenases are described in connection with tissue respiration, in Chapter 19.

- (2) *Oxidases* catalyse oxidations in presence of free oxygen which is simultaneously reduced to hydrogen peroxide.
- (3) *Peroxidases* transfer oxygen in active form from peroxides.
- (4) *Catalase*, though not an oxidising enzyme, is usually included with the oxido-reductases as it has the power of decomposing hydrogen peroxide into water and molecular oxygen.

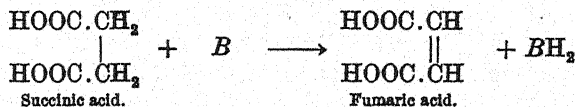
Representative Oxido-reductases

Enzyme.	Substrate.	End-product.
Glyoxalase.	Methyl glyoxal	Lactic acid.
Xanthine oxidase.	Hypoxanthine.	Uric acid.
Aldehyde oxidase.	Xanthine.	Uric acid.
	Aldehyde.	Acid.
Cytochrome oxidase.	Reduced cytochrome.	Oxidised cytochrome.
Diaphorase.	Reduced co-enzymes.	Oxidised co-enzymes.
Tyrosinase.	Tyrosine.	Melanin.
Carboligase.	Pyruvic acid.	Acetylmethyl carbinol.
Lactic dehydrogenase.	Lactic acid.	Pyruvic acid.

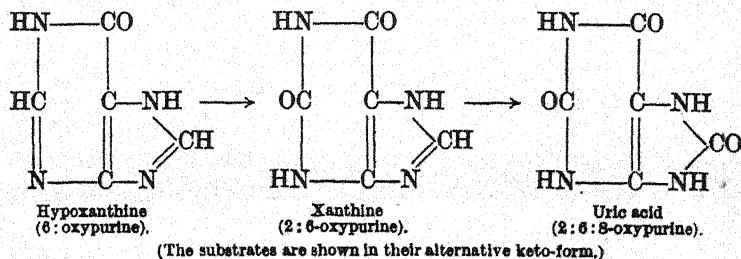
Glyoxalase, found in liver, yeast, and many tissues, converts methyl glyoxal to lactic acid, in presence of glutathione, its co-enzyme.



Succinic dehydrogenase is active in most animal tissues, and may be obtained by extracting previously-washed muscle tissue with alkaline phosphate solution. It catalyses the oxidation of succinic acid into fumaric acid in presence of atmospheric oxygen, or a hydrogen acceptor, such as methylene blue.



Xanthine oxidase oxidises the purines *hypoxanthine* and *xanthine*, and is concerned in the formation of uric acid :—

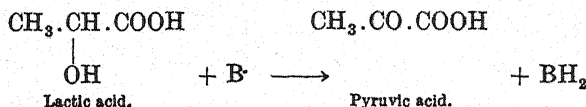


Aldehydes as a class are also oxidised by xanthine oxidase.

The enzyme is very irregularly distributed. It is completely absent from skeletal and cardiac muscle of all species examined. The liver is usually rich, except in the dog, hedgehog, and pigeon, from which it is absent. It is present in cow's milk, from which source it has been separated by Dixon.

Uricase, or *uric acid oxidase*, converts uric acid to allantoin (p. 404). The enzyme occurs in the livers of nearly all mammals, and in the kidneys of oxen, pigs, dogs, rats, and frogs.

Lactic dehydrogenase, in presence of methylene blue or other hydrogen acceptors, oxidises lactates to pyruvates.



It appears to be specific for α -hydroxy acids, converting them into the corresponding keto acid.

Lactic dehydrogenase occurs freely in animals and plants, its principle sources being yeast.

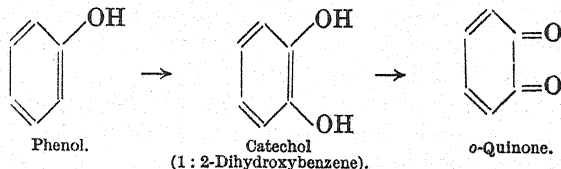
Oxidases catalyse oxidations in presence of free oxygen. Unlike the dehydrogenases, they are unable to use methylene blue, cytochrome or similar hydrogen acceptors, and they also differ in their sensitivity to inhibitors. Thus, urethane and related narcotics have no action on oxidases, but cyanide, carbon monoxide or hydrogen sulphide in concentrations as low as 0.01 M to 0.001 M inhibit them completely. This is ascribed to the fixation of an active metallic radicle, usually Fe, in the oxidase effector mechanism.

Cytochrome oxidase or **indophenol oxidase** is widely distributed in animal and plant tissues, especially those rich in nuclei, and usually accompanies cytochrome in distribution. It is absent from peripheral nerve tissue. The enzyme brings about the re-oxidation of cytochrome which has become reduced by accepting hydrogen from a substrate activated by a dehydrogenase.

In 1885, Ehrlich showed that animals after injection of a mixture of *p*-phenylenediamine and α -naphthol, developed a blue colour in their tissues, owing to formation of an indophenol pigment. Batelli and Stern, in 1912, found that this property was common to almost all mammalian tissue, and ascribed it to the presence of an enzyme, indophenol oxidase. Keilin, in 1929, discovered the significance of the enzyme when he showed that it was able to re-oxidise cytochrome, and hence forms part of an important oxidation system in the living cell.

Monophenol oxidase or **tyrosinase** occurs in all animals and in many plants, notably clover, potato, and higher fungi. It oxidises phenol, *p*-cresol, tyrosine and other monohydroxy phenols, usually

to *o*-dihydroxy phenols, or catechols, which, in turn, give rise to quinones and condensation products, as in the formation of melanin from tyrosine (p. 227).



Tyrosinase is believed to function in the formation of natural pigments from tyrosine, and also in the metabolism of the amino acid in lower organisms.

Onslow (1915) has shown that skin and hair pigmentation depends on melanin formation by tyrosinase. In *albinism* (recessive whiteness) there is a complete failure to inherit the enzyme; in dominant whiteness an enzyme inhibitor is present which prevents the formation of melanin.

Polyphenol oxidase, catechol oxidase, or laccase, which is found in most plant tissues, effects the oxidation of *o*- and *p*-dihydroxy phenols and triphenols, but does not attack monophenols, tyrosine or *p*-phenylene diamine. It resembles cytochrome oxidase in being a metallo-protein, and is inhibited by CN^- . Polyphenol oxidase is estimated colorimetrically by its yield of a violet pigment, *purpurogallin*, from pyrogallol, under standard conditions. The guaiacum colour reaction of fresh plant tissue is ascribed to oxidation of plant phenols by local oxidases, with production of *o*-quinone, which oxidises guaiaconic acid in the guaiacum to a blue pigment. Keilin has shown that Cu is the characteristic metal in polyphenol oxidase.

Peroxidases effect oxidations by causing hydrogen peroxide to transfer active oxygen to the substrate. Hæmatin derivatives and some other metallo-organic compounds are heat-stable peroxide catalysts, and are able to produce pigments from phenols and aromatic diamines (pyrogallol, guaiacol, benzidine, amidopyrine) in presence of hydrogen peroxide. This reaction is the basis of several tests for blood pigment. The position of peroxide in the living cell is obscure, as it is a powerful tissue poison. However, the majority of aerobic tissues contain both heat-labile peroxidases (enzymes) and heat-stable peroxide activators.

Rich sources of peroxidase are horse radish and potato tuber. Mammalian peroxidase is found chiefly in spleen and lung. There is a little in liver and kidney, but none in most other tissues.

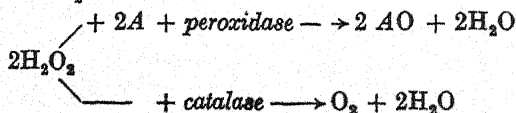
Mutase.—Dixon and Lutwak-Mann (1937) have shown that two distinct enzymes are capable of catalysing the oxidation of

aldehydes: (i.) *Aldehyde oxidase* (xanthene oxidase), which converts both aliphatic and aromatic aldehydes into the corresponding acid; and (ii.) *aldehyde mutase* (Parnas enzyme), which catalyses the Cannizzaro reaction, or mutation of two molecules of aldehyde into one of alcohol and one of acid.

Aldehyde oxidase occurs in milk, and requires no co-enzyme; aldehyde mutase is absent from milk, but well represented in muscle and liver, and requires co-dehydrogenase I (co-enzyme I).

Catalase.—A ubiquitous and important enzyme found in all aerobic tissues. It catalyses the decomposition of hydrogen peroxide into water and molecular oxygen, thus protecting the tissues from the effects of hydrogen peroxide produced during aerobic oxidations. Catalase has been isolated as a crystalline hæmoprotein from beef liver by Sumner (1937).

Peroxidase differs from catalase in that it only decomposes hydrogen peroxide in presence of an oxidisable substrate, or oxygen acceptor, *A*, and thus functions as an oxidising enzyme. Like catalase, it is a hæmatin derivative, and therefore inactivated by HCN, CO or H₂S.



Efficiency of the Enzyme Mechanism.—Haldane has calculated that at 0° C. one molecule of catalase effects the decomposition of about 2×10^6 molecules of H₂O₂ per second.

He observes that although enzymes in the cell are, in general, acting neither at their optimal temperature, pH, nor substrate concentration, it would seem that an enzyme molecule commonly must transform 100 or more molecules per second.

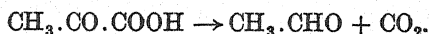
“As a very active cell, such as baker’s yeast, metabolises 10⁻⁶ of a gram molecule of O₂ per gram dry weight per second, we can get some idea of the possible complexity of the transformation undergone in it. If 5 per cent. of the dry weight of the cell consists of enzymes of an average molecular weight of 50,000, then 1 gm. dry weight contains 10⁻⁶ gm. molecule of such enzymes, and less than 1 per cent. of this would be required for any particular process, e.g., the activation of O₂. In other words, the average atom on its metabolic path through the cell may be dealt with by more than 100 catalysts in succession.”

DESMOLASES

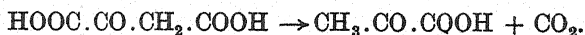
Desmolases attack the inter-carbon linkage, and thereby effect the cleavage of organic compounds during tissue respiration, muscle glycolysis, sugar fermentation, and similar events intimately associated with the continuous maintenance of life. Most of the

desmolases are endo-enzymes, and occur as members of complex systems, accompanied by other enzymes, co-enzymes and activators.

Carboxylase, de-carboxylase, occurs freely in plants and animal tissues. It decarboxylates ketonic acids by liberating CO_2 from $\text{RR}'\text{CH}.\text{CO}.\text{COOH}$, yielding the aldehyde $\text{RR}'\text{CH}.\text{CHO}$, convertible by *glyoxalase* to the corresponding acid, $\text{RR}'\text{CH}(\text{OH}).\text{COOH}$. Vegetable carboxylase, such as occurs in yeast, only activates α -ketonic acids, converting them into the corresponding aldehyde, by the reversible reaction :

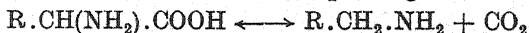


Oxaloacetic carboxylase from animal tissue converts its substrate into pyruvic acid by a similar type of reaction :—



Carboxylases are responsible for the ultimate liberation of CO_2 in all biological oxidations, and the assimilation of CO_2 by plant and animal tissue. Their activity requires the presence of the co-enzyme, *co-carboxylase* or vitamin B_1 pyrophosphate, with Mn^{++} or Mg^{++} as activator.

Amino acid decarboxylases, from animal tissue, convert tyrosine, tryptophane or histidine into the corresponding amine :



Carbonic anhydrase, although not a desmolase, decarboxylates carbonic acid, and catalyses the converse hydration of carbon dioxide, thus controlling the rate at which the gas leaves the pulmonary circulation (p. 63).

The enzyme, discovered by Meldrum and Roughton, is almost completely restricted to the red blood cells, brain cortex, gastric mucosa, and the pancreas, where it may aid in the production of the alkaline pancreatic secretion.

It is a protein complex containing 0.33 per cent. of zinc.

Detection of Carboxylase in Yeast.—Place 1–2 ml. on a 1 in 5 yeast suspension in a Petrie dish or in the outer compartment of a Conway unit. Place about 1 ml. of saturated $\text{Ba}(\text{OH})_2$ solution in a watch-glass resting in the dish, or in the central compartment of the unit. Add 1 ml. of 1 per cent. pyruvic acid to the yeast suspension, and cover the dish or unit. Yeast is very rich in carboxylase, which acts on the pyruvic acid, releasing CO_2 , which diffuses into the barium solution, and forms a white precipitate of BaCO_3 . A control test, omitting the pyruvic acid, should be carried out simultaneously.

EXTRACTION AND PURIFICATION OF ENZYMES

No general method of enzyme preparation is available. Some, the desmo-enzymes, are restricted to the interior of cells ; others, the

lyo-enzymes, escape into secretions and culture fluids. Purification requires removal of living organisms and inert cellular constituents, including proteins and other colloids likely to adsorb the enzyme. Four methods are in use :—

(1) Precipitation with alcohol or acetone. All enzymes are insoluble in alcohol, and may be precipitated on addition of excess. The volume required depends on the nature of the enzyme and the impurities present. Thrombokinase is precipitated by 10–15 per cent.; rennin requires 80–90 per cent.

In 1926, Sumner isolated urease, the first enzyme obtained in crystalline form, by the simple process of extracting fat-free jack bean flour in 31.6 per cent. acetone, and allowing the filtrate to crystallise at 0° C. Crystalline catalase has been got in a similar manner by dioxan precipitation of liver extract (Sumner and Dounce, 1935).

(2) Fractional "salting-out," by addition of ammonium sulphate or similar salt, at a suitable pH. This method has been used successfully by Northrop in isolating pepsin and other proteinases.

(3) Specific adsorption. Enzymes showing acidic properties can be removed from solution by addition of an insoluble basic adsorbent, usually aluminium hydroxide. Basic enzymes can be removed by use of an acidic adsorbent, usually kaolin. The adsorbed enzyme is liberated, or eluted, from the precipitate by changing the pH of the mixture or by addition of a more acceptable adsorbent.

Alumina adsorbs acid dyes, crude pancreatic lipase, pancreatic peptidase, plant peroxidase, and other acidic enzymes. Kaolin adsorbs basic dyes, trypsin, and other basic enzymes. Pancreatic amylase is not adsorbed by alumina or by kaolin, hence by the successive use of these adsorbents the three chief enzymes in pancreatic juice may be separated.

Chemical Nature of Enzymes.—At least thirty enzymes or enzyme-precursors have now been obtained as crystalline proteins. They include: *urease*, *catalase*, *pepsin*, *trypsin*, *chymotrypsin* (the milk-coagulating enzyme of the pancreas), *pepsinogen*, *trypsinogen* and *chymotrypsinogen*, pancreatic *carboxy-peptidase*, *papain*, *amylase*, *lysozyme*, *lactic dehydrogenase* and *phosphorylase*. Others obtained in conditions of approximate purity are: *sucrase*, *peroxidase*, *lipase* and *xanthine oxidase*.

From variations in the composition of their preparations Willstätter and Waldschmidt-Leitz have been led to a *bearer* theory of enzyme structure, according to which each enzyme consists of (1) a colloidal bearer, usually of protein nature, and (2) one or more active groups which enable the bearer to become affixed to the substrate. The distinction between the soluble lyo-enzymes and the insoluble desmo-enzymes is determined by the solubility of the protein bearer. Sumner and Northrop, however, maintain that the crystalline enzymes are pure proteins, and that even partial hydrolysis of the protein destroys the enzyme. While, of

course, it is possible that both theories may be true in that they apply to different enzyme types, the balance of evidence supports the conclusions of the American workers, and leads to the recognition of a new class of biochemical compounds, the *zymo-proteins*.

Mechanism of Enzyme Action.—Two processes occur in zymolysis : (a) adsorption of the substrate by the enzyme, (b) activation and transformation of the substrate while bound to the enzyme surface. That zymolysis is a surface or adsorption phenomenon is shown by the fact that the change does not proceed entirely in accordance with the simple law of mass action in respect to both reactants.

By increasing the concentration of the enzyme, the rate of reaction is increased over a wide range, but when the concentration of enzyme is kept fixed while the concentration of the substrate is increased a point is reached when further addition of the substrate does not increase the rate of the reaction. When this point is reached, the enzyme surface is saturated with substrate, and remains saturated as long as excess of substrate remains in the mixture. Union between enzyme and substrate is determined by the presence of effector groups asymmetrically arranged in the "binding-plane" of the enzyme surface, and uniting with reciprocal groups in the binding plane of the substrate.

The pattern of these groups in space decides the specificity of the enzyme for a substrate or substrate type. Thus, many enzymes including hexases and peptidases have an antipodal specificity in that they will not attack the optical isomers of their substrates.

The substrate molecule must fit exactly into the mosaic pattern of the enzyme surface. The combination is only possible if enzyme and substrate can approximate in such a way that the two hypothetical binding-planes are not more than a few Ångström units apart (Bergmann, 1937). Thus, pancreatic peptidase will not attack synthetic peptides made from the non-natural D-amino acids.

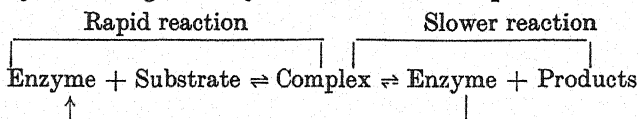
The chemical nature of the effector groups depends on the substrate attacked. Dipeptidases and amino-peptidases combine with a free amino group in their substrates, possibly by means of an aldehyde group. The activity of papain, cathepsin, urease, and perhaps arginase, depends on their state of oxidation-reduction as determined by the $R-S-S-R + 2H \rightleftharpoons R-SH + HS-R$ equilibrium of their effector groups.

Substrate Activation.—According to the "Two-affinities" theory of von Euler, the substrate is attached to the enzyme by more than one effector group and the resulting strain set up in the molecule leads to its rupture, when the fragments no longer held at two points escape into the body of the solution and are replaced by a fresh substrate molecule. Quastel has tabulated a number of organic

genation of pyruvic acid, $\text{CH}_3\text{CO}\cdot\text{COOH}$, is inhibited by glyoxylic, $\text{H}\cdot\text{CO}\cdot\text{COOH}$, and by oxalic acid, $\text{HOOC}\cdot\text{COOH}$. Dehydrogenation of succinate is inhibited by malate (p. 375).

Rates of Catalysed Reactions.—No simple general law is known that relates the three variables: enzyme concentration, substrate concentration, and rate of reaction under constant conditions of temperature and pH. Three cases may be recognised: (1) where the substrate concentration is so high that the enzyme is saturated during the greater part of the zymolysis; (2) where the substrate does not saturate all the enzyme; and (3) where the substrate concentration changes so much during the reaction that the degree of enzyme saturation falls from a maximum to a minimum.

The Michaelis Equation.—In 1913, Michaelis proposed an equation that has been of value in grading enzymes in terms of their “affinity constants,” K_m , for given substrates. The equation assumes that the first stage in an enzyme reaction is a very rapid union of enzyme and substrate as an unstable complex, which then decomposes, less rapidly, liberating the enzyme and the reaction products.



Let E = Concentration of enzyme, both free and bound in the complex,

C = Concentration of the enzyme-substrate complex,

S = Concentration of free substrate, which is usually much greater than E .

Then $(E - C) =$ Concentration of free enzyme.

At any instant, by the law of mass action, the concentration of free enzyme multiplied by the concentration of free substrate is proportional to the concentration of the enzyme-substrate complex, or $(E - C) \times S = K_m C$,

where K_m is a constant for the particular enzyme system.

Then, $K_m C = ES - CS$, and $C = \frac{ES}{K_m + S}$ (1).

Since the speed of formation of the complex is assumed to be almost instantaneous, the rate of catalysis, v , is determined by the rate at which the complex decomposes.

Hence, v is proportional to C , or $v = K_c C$, where K_c is the velocity constant for the decomposition of the complex.

Substituting in equation (1), $v = \frac{K_c \cdot ES}{K_m + S}$ (2).

Catalysis proceeds at its maximum speed, V , when conditions are such that all the enzyme is bound to the substrate.

Here, $E - C = 0$, or $E = C$; and $V = K_c C = K_c E$.

Substituting in equation (2), the *Michaelis equation* is obtained :—

$$v = \frac{VS}{K_m + S}, \text{ or, } K_m = S \left[\frac{V}{v} - 1 \right],$$

where v is the observed rate of catalysis, V the maximum possible rate, S the concentration of the substrate, and K_m the constant for the particular enzyme-substrate system.

The rate of the reaction, v , can be measured for different known values of S . When v is half V , the maximum rate, the constant K_m becomes numerically equal to S , since here $K_m = S(2-1) = S$. This has been verified experimentally.

K_m , the Michaelis constant, has been found for various enzyme systems, and may be defined as the value of the substrate concentration necessary to give half the maximum rate of zymolysis.

K_m is large for the hydrolase enzymes (0.01 M to 0.1 M), and small for the oxidases (0.0001 M to 0.01 M). The affinity of an enzyme for a particular substrate is thus inversely proportional to its K_m value for that substrate system.

The Michaelis equation has been criticised by Briggs and Haldane (1925), who object to the assumption that the speed of formation of the enzyme-substrate complex is infinite when compared with the observed speed of the reaction. They have proposed a modified equation in which K_m is replaced by $(K_2 + K_3)/K_1$, where K_1 is the speed of complex formation, K_2 the speed of its reversion into enzyme and substrate, and K_3 the speed of its decomposition into enzyme and products.

Working with pure enzymes, Northrop has, in general, confirmed the principles of the Michaelis equation.

Alternative theories of enzyme mechanism and reaction rates are proposed by D. B. Taylor (1938), Wilson (1939) and Sumner (1943).

Reversible Catalysis.—A catalysed reaction comes to rest when the end-products have accumulated to such an extent that they are able to compete as effectively as the remaining substrate for possession of the surface of the enzyme. This is a typical condition of dynamic equilibrium.

The equilibrium level differs for different conditions. In ester systems, zymolysis usually ceases when about three-quarters of the original substrate is hydrolysed. In the dilute urea-urease system,

zymolysis continues until about 99 per cent of the urea is decomposed, and if the liberated ammonia be removed by aspiration the reaction continues to completion.

The same equilibrium level can be reached from either side of the reaction. If the enzyme be added to a concentrated mixture of the end-products, **reversion synthesis** takes place with formation of sufficient of the original substrate to make an equilibrium mixture with the end-products. Such reversion is of great biological importance, and is believed to be the mechanism whereby most animal and plant syntheses are brought about. Reversion is intracellular; the immobile enzymes are continually removing soluble metabolites and converting them into less soluble tissue constituents. For this reason, many enzymes and their natural substrates are found associated in nature. Thus, lipase occurs in seeds rich in oil, glucosides and glucosidases are present in plants, hippuricase accompanies hippuric acid in the kidney. This process requires additional factors to enable the reversion synthesis to continue, otherwise the synthetic products could not accumulate to the extent normally found in tissues. Some agent is at work removing the surplus reversion products as they are formed. This may be an adsorption mechanism in the cell or some associated enzyme. Serial reactions are of common occurrence in biology, and it is probable that an individual reversion synthesis is only one rather obvious link in a long chain.

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CHAPTER 14

NUTRIENTS

" ' All flesh is grass ' is not only metaphorically but literally true ; for all those creatures we behold are but the herbs of the field, digested into flesh in them, or more remotely carnified in ourselves ; . . . this frame we look upon hath been upon our trenchers ; in brief, we have devoured ourselves."

THOMAS BROWNE.

NUTRITION is the process whereby an organism incorporates material from its environment for the purposes of growth, maintenance and repair. A nutrient is a substance capable of being assimilated and utilised by an organism.

Foodstuffs are natural or manufactured products of plant or animal origin, used as sources of nutrients.

Classification.—Nutrients may be classified : (1) *chemically*, according to structure, such as carbohydrates, proteins, metals ; (2) *physiologically*, according to the processes involved in their digestion and assimilation. Direct nutrients, such as water, simple sugars and amino acids, are rapidly absorbed, and thus are of value in emergency conditions. Indirect nutrients, such as proteins and fats, require the operation of the digestive process. (3) A *biochemical* classification can be made of (a) *constructive nutrients*, which provide components of tissues, reaction systems and secretions ; and (b) *combustible nutrients*, which provide energy for the maintenance of body temperature and the chemical traffic of life.

Constructive nutrients include water, inorganic ions, the 8-10 indispensable amino acids, the vitamins or pro-vitamins, and some miscellaneous compounds, including choline, and polyene fatty acids. These, and probably some not yet identified compounds, are required by the human body. Other species differ in their exact needs.

Combustible nutrients include carbohydrates, the non-essential parts of the proteins, aliphatic acids and glycerol, from fats. Oxygen provides the energy of the animal, by uniting with the hydrogen transferred from organic compounds.

All combustible nutrients may be assessed in terms of their energy content, usually measured in kilocalories per gram of dry material.

Man, like other animals, resembles an internal combustion engine designed to run at a moderate temperature. Like a producer-gas unit, he can deal with a variety of different types of fuel, always provided that he has sufficient to keep the engine running continuously, for Nature has not equipped him with a self-starter.

The energy requirement of the average adult is assessed, popularly, but not always practically, at 3,000 kilocalories for twenty-four hours. A more accurate scale has been drawn up by the Health Committee of the League of Nations, in 1936, according to which a normal adult, living an ordinary life in a temperate climate, and not engaged in manual work, requires a daily basic supply of 2,400 kilocalories. This is supplemented according to individual needs, additions, in terms of kilocalories *per hour*, being: light work, 75; moderate work, 75-100; hard work, 150-300, and upwards. The special requirements of children and pregnant or lactating women are calculated by multiplying the basic supply by an appropriate coefficient. Children under one year require a daily intake of about 100 kilocalories *per kilogram* body weight. Above that age, the scale of daily requirement is:—

Years .	1-2	2-3	3-5	5-7	7-9	9-11	11-12	12-15
Kilocal. .	840	1,000	1,200	1,440	1,680	1,920	2,160	2,400

The calorie demand for growth is higher than is generally realised, and explains the hunger for carbohydrates, characteristic of healthy childhood. The calories of the human mixed diet are derived from the organic nutrients, the usual partition being: carbohydrates, 55-66; proteins, 10-15; lipides, 20-35; expressed as percentage contribution to total calorie intake.

The normal healthy adult, of 65-70 kgm. weight, using about 36 kilocalories *per kgm.* in twenty-four hours, excretes 2,300-3,600 gm. of H_2O , and 470 litres of CO_2 , which is equivalent to 250 gm. of carbon consumed during the same period.

CHARACTERISTICS OF THE FOOD MATERIALS

(1) **Oxygen.**—Oxygen is a unique nutrient. It is the only material accepted by the higher organism as a free element and in the gaseous form; it interacts with, and liberates energy from, the organic food materials; and it is not assimilated by means of the alimentary tract.

So important is the continuous provision of oxygen that a special apparatus, the respiratory system, has been evolved for this purpose.

The adult human lungs have a volume of about 3 litres, and normally contain about 0.5 litres of oxygen, which suffices for the body at rest for about two minutes. If the supply is not maintained, a condition of anoxæmia develops rapidly, and death follows acute oxygen starvation in about three minutes.

(2) **Water.**—Water is not regarded popularly as a food material because it yields no energy to the organism. It is, however, the chief constituent of most of the tissues, and thus contributes to the structure of the body.

The total water intake of the normal adult is between 2–3.5 litres *per diem*, more than half of which is eaten in the form of solid food.

A 3,000-kilocalorie diet as consumed contains about 2,000 ml. of preformed and potential water and must be supplemented by the further consumption of 1 to 1.5 litres in a liquid form.

A satisfactory daily water intake for the adult is 1 ml. per kilocalorie of food.

Circumstances of modern marine warfare have shown that 500 ml. (18 oz.) represent the smallest daily supply of water capable of maintaining life in occupants of boats adrift.

*Average Water Content of Prepared Foodstuffs
Expressed in Percentage*

Foodstuff.	Water.	Foodstuff.	Water.
Fruits	75–92	Eggs	73
Green vegetables	74–97	Cheese	23–60
Milk	83–87.5	Bread	24–40
Meat (cooked)	48–77	Butter	17
Potatoes (boiled)	76	Biscuits.	5–10

The amount of water consumed has a minimal limit fixed by the osmotic tension of the circulating fluids. When the concentration of certain solutes, notably Na^+ and Cl^- , rises above the normal plasma level the sensation of thirst is evoked.

(3) **Metallic Ions.**—The biological metals are widely and unequally distributed in the dietary. Some of them occur as organic compounds, the nature and fate of which are obscure, but which are believed to undergo decomposition by the acid and alkali of the alimentary tract. Others occur as simple derivatives of chloride, carbonate, sulphate, and phosphate, the assimilable units are, presumably, the ions.

The exact human requirement of many of the individual metals is known, approximately, from analyses of satisfactory diets, and the need is conveniently expressed as (1) *minimal demand (M.D.)*,

below which health cannot be maintained, and (2) *optimal demand* (O.D.), which provides a safety margin.

Inorganic Nutrients

Element.	Adult Daily Requirements.		Chief Sources.
	M.D.	O.D.	
Na ⁺	1.0 gm.	1.5 gm.	Table salt, animal products.
K ⁺	1.0 gm. (?)	3.5 gm.	Vegetable and animal products.
Ca ⁺⁺	0.75 gm.	1.5 gm.	Cheese, milk, egg yolk, "hard" water.
Mg ⁺⁺	0.2 gm. (?)	0.5 gm.	All green vegetables.
Fe ⁺⁺	10 mg.	20 mg.	Liver, treacle, egg-yolk, lentils, parsley, cocoa, cereals, dried currants and raisins, lean meat.
Cu ⁺⁺	0.1 mg. (?)	3 mg.	Liver, cocoa, chocolate, oysters, mushrooms, treacle, nuts, cereals, egg-yolk.
Mn ⁺⁺	+	1 mg. (?)	Liver, kidney, pancreas, bran, tea, green vegetables, lean meat.
Co	+	+	
Zn	+	+	
Cl ⁻	7.0 gm. (?)	7.0 gm.	As for Na ⁺ .
S	0.5 gm. (?)	1.0 gm.	Cystine and Methionine, from cheese, milk, egg-yolk, and other protein sources.
P	1.0 gm.	1.5 gm.	Phosphoproteins and phospholipides from cheese, milk, egg-yolk, liver, meat.
as PO ₄ ≡	3.0 gm.	4.5 gm.	Crude table salt containing NaI, fish, liver and kidney.
I ⁻	0.05 mg. (?)	0.2 mg.	Drinking water.
F ⁻	+	+	

(4) **Carbohydrates.**—About 60 to 70 per cent. of the solids of the human diet are carbohydrates, represented by four sugars (glucose, fructose, lactose, and sucrose) and, chiefly, by the polysaccharide starch.

Glucose and fructose occur in syrups, honey, sweet fruits and preserves. Sucrose, obtained from sugar cane (13 per cent.) or sugar beet (15 per cent.), is the common sweetening agent, although of late it is being supplemented by glucose (starch sugar) in the manufacture of preserves.

Lactose is obtained from milk (3–4 per cent.), but, except in the diet of children, the amount consumed is small.

Glycogen is usually converted to maltose before it is consumed, unless it be eaten in the form of raw oysters, fresh meat or liver (1 per cent.).

Starch, the chief carbohydrate of the diet, occurs in cereals (wheat, oats, barley, rice, Indian corn, rye), where it reaches the high concentration of about 70 per cent. It is present also in tubers and roots (potato, 18–20 per cent.; parsnip, carrot, turnip, 3–11 per cent.;

arrowroot, sago, tapioca). Bread contains from 45 per cent. (whole-meal) to 55 per cent. (white).

Cellulose, or vegetable fibre, does not contribute energy, since it is not attacked by any enzyme of the human alimentary tract. It may, however, have some service as a mechanical aid to peristalsis, and in the form of bran and under the name of "roughage" it has been prescribed, if not consumed, with enthusiasm.

Calorific Value.—Carbohydrates have a heat equivalent of about 4 kilocalories per gram.

Starch, being less hydrated, is 4.14; disaccharides are 3.9; and monosaccharides about 3.7.

Since 400–500 gm. of carbohydrate may be consumed in the ordinary daily dietary, it will be seen that it contributes about two-thirds of the total energy requirement of the adult. Carbohydrates represent the cheapest food materials, and for this reason they predominate in the dietaries of the poor and the parsimonious.

(5) **Proteins.**—The nitrogenous food materials make up 10–15 per cent. of the solids of the human diet, and may reach 30 per cent. if the consumer can afford them. They are the most expensive and most palatable part of the dietary, and are well represented in lean meat and fish (18–20 per cent.), cheese (10–40 per cent.), egg white (12 per cent.), egg yolk (16 per cent.), liver (29 per cent.), kidney (26 per cent.), milk (3–4 per cent.), bread (6 per cent.), potato (1–2 per cent.), green vegetables (1–2.5 per cent.), beans and peas (6–20 per cent.). Proteins form the only dietary source of amino acids, the content of which differs in different proteins. For this reason, it is generally believed that about half of the food protein should be derived from animal sources to ensure getting a sufficient supply of the indispensable acids, and several sorts of protein are included in the dietary so as to provide an adequate selection.

Calorific Value.—When completely combusted into carbon dioxide, nitric acid, and water, 1 gm. of dry protein yields 5.8 kilocalories of energy. In the organism, however, oxidation of the nitrogen is only carried as far as urea, and for this reason the calorific value of food protein is only 4.1 kilocalories per gram.

The higher animal is an extravagant apparatus. It has evolved unequally, and has lost the power of synthesising many of the organic compounds necessary for its existence, and must depend on its dietary for these exogenic factors. In consequence, its food supply is varied and complex, and requires a series of digestive juices, which, together with the mucin secreted by the alimentary tract, make up much of the 2 to 3 gm. of nitrogen lost daily by the intestine.

Protein Requirements of Man.—The optimal requirement for the human subject is rated at 1 gm. protein per kg. body weight, *per diem*. This allowance is made on the assumption that the protein of the diet is mixed and of a good quality. The protein intake must provide a sufficiency of the essential amino acids, of which at least eight species are necessary: tyrosine or phenylalanine, tryptophane, methionine, lysine, threonine, leucine, isoleucine, and valine. Digestibility must also be taken into account, and it is now customary to assess proteins in terms of their *biological value* to the consumer.

Children in addition require arginine and, possibly, histidine, if they are to maintain their proper growth-rate.

The total daily need of children, expressed in gm. protein per kgm. body weight, are :—

Year . . .	1-3,	3-5,	5-15,	15-17,	21 upwards.
Protein . . .	3.5,	3.0,	2.5,	1.5,	1.0

The total daily requirement of the lactating mother is 2 gm. protein per kgm. body weight.

Protein requirement is calculated from nitrogen-balance values, and represents the intake of protein nitrogen sufficient to maintain normal growth rate in young animals, or to equal the total nitrogen excreted by the adult in a state of constant body weight.

Health has been maintained in adults on protein daily intakes as low as 30 gm. (Chittenden, 1908) and 35 gm. (Kon and Klein, 1928). While Hindhede, of Copenhagen, has kept himself in nitrogen-equilibrium and health on a 2,650-kilocalorie diet containing only 6 gm. of protein. These achievements demand animal protein

(such as milk casein), rich in all the indispensable amino acids, and, also, the acquirement, through training, of a biological economy by the consumer. Serious dietary protein deficiency is revealed by subnormal growth, decreased resistance to infective diseases, and nutritional oedema, due to insufficient protein in the plasma to keep water from escaping into the tissues.

The *biological value* of a protein in the diet may be calculated from (1) growth-rate effect, or (2) balance-sheet data for nitrogen intake and output (H. Chick, 1942).

The *biological value* of a protein *X* is expressed as :—

$$100 \times \frac{\text{Body nitrogen saved}}{\text{Food nitrogen absorbed}} = 100 \times \left\{ 1 - \frac{UN_x - UN_e}{IN_x - (FN_x - FN_e)} \right\}$$

where UN_x = Urinary Nitrogen excreted on a diet containing X ,

UN_e = Urinary Nitrogen excreted on a nitrogen-free diet,

IN_x = Food Nitrogen intake in the form of X ,

FN_x = Faecal Nitrogen excreted on a diet containing X ,

FN_e = Faecal Nitrogen excreted on a nitrogen-free diet.

UN_e and FN_e represent the endogenous nitrogen output, which is assumed to be constant irrespective of the diet. The maximum biological value of a protein on this scale is 100, which indicates the utilisation of all the absorbed food nitrogen.

With the exception of the milk proteins, the biological value of which remains high at all levels, increasing the proportion of protein in the diet from 3 to 5, 7 or 10 per cent. lowers the biological value.

Biological Value of Proteins (Chick et al., 1935)

Protein	Low Level Intake.		High Level Intake.	
	Dietary percentage.	Biological Value.	Dietary percentage.	Biological Value.
Wheat, entire .	3.6	100	5.6	68
Wheat, germ .	3.6	90	6.8	69
White flour .	3.1	84	7.0	61
Milk, dried .	3.4	89	6.9	81
Lactalbumin .	3.1	92	6.7	65

The average daily protein intake of 80–100 gms. provides about 400 kilocalories of energy, leaving a balance of some 2,600 kilocalories to be supplied by the lipides and the carbohydrates.

(6) **Lipides.**—These include the true fats and the lipines, although the latter are usually neglected in nutritional calculations. The true fats make up about 10–20 per cent. of the diet, depending on the food habits and season of the year.

Their chief sources are : (1) *animal fats*, lard, suet, butter (83 per cent.), cream (20–40 per cent.), milk (3–4 per cent.), egg-yolk (21–31 per cent.), meat (1–20 per cent.), mackerel and herring (6–8 per cent.), salmon (12 per cent.). (2) *Vegetable fats*, oils, oleo-margarines (84 per cent.), nuts (35–60 per cent.). Phosphatides come mostly from the lecithins or egg-yolk, liver and kidney. Fats are important as concentrated sources of energy, and represent a form in which energy is stored in animals and in the seeds of plants.

Calorific Value.—On account of their low oxygen content, fats have the high energy value of 9.3 kilocalories per gram.

The Lipide Requirements.—The lipides of the dietary differ from the other foodstuffs in that they carry as solutes various highly important micro-essential nutrients, including vitamins A and D

and the provitamin carotinoids and sterols. The complex lipides also provide choline, the anti-liposis factor. The average consumption of lipide in temperate countries is of the order of 1 gm. per kg. body weight, *per diem*, and is ingested both as animal and vegetable fats. This intake varies with circumstances and seasons, but provides about 600 to 700 kilocalories *per diem*. Fat metabolism requires to be balanced by carbohydrate, and if the fat-content of the dietary exceeds a limiting value, a ketosis may occur, due to the a cumulation of aceto-acetic acid, and acetone. This ketogenic state cannot arise under dietary conditions where twice the total carbohydrate intake together with half the protein intake are greater in weight than the total fat intake.

VITAMINS AND PROVITAMINS

Vitamins are organic micro-constituents of the diet, and are necessary for the growth and maintenance of animals. They may be regarded as *exogenous* hormones which the organism is unable to manufacture for itself; and like the autacoids or *endogenous* hormones of animal origin, they are therapeutic agents of great potency as well as determinants of normal growth and activity. Vitamins are classified provisionally according to solubility and chief therapeutic effect.

The Vitamin Theory.—The acceptance of the vitamins as an independent class of food constituents is due to the accumulation of evidence from five distinct sources: (1) the clinical recognition of rickets, scurvy, and beri-beri as deficiency diseases; (2) the empirical use of natural products rich in vitamins, such as liver oils, lemon juice, and yeast; (3) the experimental proof that animals are unable to live and grow on dietaries of purified protein, carbohydrate, lipide, and inorganic salts; (4) the production and cure of typical deficiency diseases; (5) the separation and synthesis of the actual vitamins.

History.—In 1873, Forster, Voit's assistant in Munich, examined the effect of a de-mineralised diet on dogs and pigeons. Using purified protein, fat, and starch, he found that the animals died within a month, and concluded that "food deprived of its inorganic salts causes death more rapidly than total deprivation of food."

In 1881, Bunge repeated these experiments on mice in order to find which salts were necessary for life. He concluded that an additional factor was required, "although animals can live on milk alone, yet if all the constituents of milk which according to the present teaching of physiology are necessary for the maintenance of the organism be mixed together, the animals rapidly die . . . does milk contain in addition to proteid, fat, and carbohydrates, other organic substances which are also indispensable to the maintenance of life?" (1902).

VITAMINS NECESSARY IN HUMAN NUTRITION

*Minimal and Optimal Daily Demand in Milligrams
or International Units*

I. Fat-soluble Vitamins

Vitamin.	M.D.	O.D.	Protects Against
A	1 mg. 3,000 I.U.	3 mg. 7,000 I.U.	Xerosis, or keratinisation of epithelial tissue.
Provitamins A	2 mg. 3,000 I.U.	5 mg. 7,000 I.U.	Night-blindness.
(β -Carotene)	0.005 mg. 200 I.U.	0.01 mg. 400 I.U.	Rickets.
D ₂ , calciferol	"	"	Defective ossification.
D ₃	"	"	Malabsorption of Ca ⁺⁺ and PO ₄ ^{'''} .
Provitamins D (ergosterol)			
E, tocopherols	? (0.1 mg. for rats)	?	Abortion. Male sterility.
K	?	?	Hæmorrhage due to lack of prothrombin.

II. Water-soluble Vitamins

B ₁ , Thiamine	1 mg. 350 I.U.	3 mg. 1,000 I.U.	Beri-beri. Pyruvic ketosis.
or aneurin			
B ₂ , riboflavin	1 mg. (?)	2 mg.	Stomatitis, glossitis, kera- titis.
Nicotinic acid or nico- tinic amide	?	10 mg.	Pellagra
B ₆ , pyridoxin or ader- min	?	?	Dermatitis, anæmia.
C, ascorbic acid.	25 mg.	75 mg.	Scurvy.
H, Biotin	0.1 mg.	?	Dermatitis.
P.	?	?	Capillary fragility.
para-amino benzoate	?	?	Sub-normal growth.
Pantothenic acid	?	?	" "
Inositol	?	?	" "
Folic acid.	?	?	Anæmia.

Hopkins, in 1906, recognised the association between these unknown food factors and the widespread occurrence of deficiency diseases: "Scurvy and rickets are conditions so severe that they force themselves on our attention; but many other nutritive errors affect the health of individuals to a degree most important to themselves, and some of them depend on unsuspected dietetic factors."

A year later, Fraser and Stanton obtained by the alcoholic extraction of rice bran a product capable of curing beri-beri, and, in 1911, Funk obtained a growth-promoting residue; since it contained basic nitrogen, it was termed a vitamin.

In an important paper, published in 1912, Hopkins showed the presence of "accessory food factors" in milk which were essential for the growth of rats. In 1915, these were resolved by McCollum and Davis into a fat-soluble A factor and a water-soluble B factor, and a third, the water-soluble C factor, was included by Harden and

Zilva, in 1919. Since then the original A factor has been resolved into A and D components, and the original B factor has been shown to be a mixture of several vitamins.

The potency of the vitamins is such that, for man, a daily dosage of the order of 0.1—5 mg. is adequate, with the exception of vitamin C, the requirement of which is 25—75 mg., and vitamins D, the requirement of which is only about 0.01 mg. Many occur naturally as *provitamins*, capable of being transformed into vitamins by the animal.

THE FAT-SOLUBLE VITAMINS

Then the Angel said to him : Take out the entrails of this fish, and lay up his heart, and his gall, and his liver, for thee : for these are necessary for useful medicines.

[Tobit, VI, 5.]

(1) **Vitamin A.**—A growth-promoting fat-soluble vitamin was located, independently by McCollum and Davis (1913) and by Osborne and Mendel (1913), in butter and cod-liver oil. It is known variously as fat-soluble A, and the *axerophthol*.

Sources.—Vitamin A occurs chiefly in storage fats of liver, such as fish-liver oils, and in the fat of milk, cream, and butter. Its precursors are found in egg-yolk, seed embryos, and, universally, in green, leafy vegetables, flowers, and fruits containing the yellow carotene pigments. The vitamin does not occur in plants, except as a precursor, which is synthesised exclusively by plants, and is stored in lipid tissues.

Properties.—Vitamin A, $C_{20}H_{29}.OH$, is an alcohol derived from semi- β -carotene, and may be replaced in nutrition by any one of the many carotenes containing the semi- β -carotene residue (α -carotene, β -carotene, γ -carotene, and cryptoxanthin). The formulæ of these compounds and vitamin A are given on pp. 222–3.

Transformation of the provitamins into the vitamin takes place in the liver, where the vitamin subsequently is stored. Vitamin A is sparingly soluble in water, but dissolves freely in fats and fat-solvents, the solutions showing the characteristic absorption-band in the ultra-violet region at 328 $m\mu$, also displayed by the vitamin itself, which when pure is a colourless oil. The vitamin is stable to heat, acids and alkalies, but is easily inactivated by oxidation, and is also destroyed during the hydrogenation of oils to form solid food fats. Preparations of A can be stabilised by small amounts of anti-oxidants, such as vitamin E and hydroquinone.

Functions.—Vitamin A is necessary for the normal growth of young animals, and for the maintenance of adult tissues of epiblastic origin (central nervous system, retina, skin and dermal glands, oral and nasopharyngeal mucosa, salivary glands).

Vitamin A thus is essential for vertebrates, all of which can convert the provitamin carotinoids into A, but there is no evidence that any vertebrate can synthesise either carotene or A.

Effects of Vitamin A Deficiency.—(1) *Failure of Growth.*—This is obvious only in young animals. The growth-rate falls off rapidly when the vitamin is withheld and the tissue-reserves have been exhausted. The animal continues to exist at a subnormal weight until secondary disturbances or infections develop.

(2) *Keratinisation of Epithelial Tissue.*—The most characteristic sign of avitaminosis A is the change seen in the skin, mucous membranes and conjunctiva. Dryness of the skin is followed by a papular eruption due to lack of secretion by the sebaceous glands of the hair follicles. This state of *phrynoderma*, or "toad skin," has been recognised in Africa and in Asia as an early form of vitamin A deficiency in human subjects.

- (i.) *Xerophthalmia*, or keratosis and ulceration of the cornea. The lachrymal secretion is diminished, and the conjunctiva becomes dry and inflamed. Secondary infections lead to ulcerations, which, by involving the anterior chamber, may terminate in total blindness.

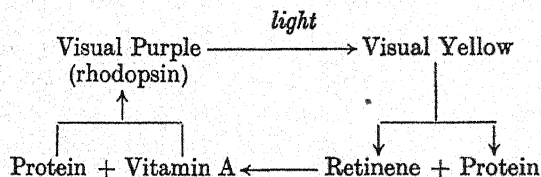
While the infection is independent of the vitamin, the predisposing syndrome is characteristic of avitaminosis A, and in the early stages yields specifically to treatment.

- (ii.) *Gastro-enteritis.*—The mucosa of the alimentary tract degenerates and the villi become necrosed. Ulceration of the stomach and intestines may set in subsequent to oral infection.
- (iii.) *Pharyngitis.*—Keratinisation of the naso-pharyngeal epithelium predisposing to inflammation of the upper respiratory tract.
- (iv.) *Nerve Degeneration.*—Mellanby (1934) claims that vitamin A is necessary for the maintenance of the nervous tissues, and that avitaminosis A is responsible for the degenerative changes seen in beri-beri, pellagra, disseminated sclerosis of the cord, lathyrism, convulsive ergotism and pernicious anæmia. The principal evidence is the beneficial effect of vitamin A therapy in the treatment of disseminated sclerosis. But Harris observes (1936): "If chronic vitamin A deficiency is the cause of the nervous lesions . . . one might expect to see evidence of its better known symptoms, such as local infections and xerophthalmia."

- (v.) *Night-blindness*, or *Nyctalopia* (hemeralopia).—Before marked clinical signs appear, an early deficiency condition can often be inferred from ocular changes leading to loss of vision in dim light, and terminating in degeneration of the retina and optic nerve. The condition is common in Greece and in Newfoundland (Aykroyd, 1936), where it has been treated, empirically, for many years by fish-liver, a prescription adopted successfully by Tobit some three thousand years ago.

Avitaminosis A manifests itself collectively as a lowered resistance to infections, and for this reason the vitamin has been called "the anti-infective factor." The term is misleading, as the vitamin has not been found to have any curative effect in actual infections, but owes its importance to its position as a tissue component.

Vitamin A and Retinal Metabolism.—The rods of the retina contain a chromoprotein, *rhodopsin*, or visual purple, which is photo-sensitive, and participates in the conversion of light energy into transmissible nervous stimuli. Light changes rhodopsin into another pigment, *visual yellow*, which is subsequently decomposed into protein and a carotinoid, *retinene*. Wald, the discoverer of the reaction, has shown that retinene is converted into vitamin A, which recombines with the protein to regenerate rhodopsin, thus forming a photo-chemical cycle in the retina.



The Rhodopsin Cycle (Wald, 1934).

Visual violet, or *iodopsin*, also a chromoprotein or vitamin A, occurs in the cones of the retina.

Vitamin A Standard and Requirements.—The international standard adopted by the League of Nations (1934) for estimating and administering vitamin A is the biological activity of 0.6 μg . of pure β -carotene, in coconut oil, stabilised by hydroquinone.

The nutritional standard originally used in America is one devised by Sherman, and represents the amount of vitamin or provitamin capable of maintaining a gain of 3 gm. a week in the weight of a young rat on a controlled diet. One Sherman A unit equals approximately 1.4 international units. The minimal daily requirement of vitamin A for the human adult, according to Fraps

and Treichler (1933), is 1,000 Sherman units, but many workers prefer to exceed this recommendation, especially in child nutrition, and advise an intake of 4,000 international units, or the equivalent of about 3-6 mg. carotene *per diem*. The most potent sources are pure carotene, halibut-liver oil (which has an actual vitamin content ranging from 0.1 to nearly 10 per cent.), salmon-liver oil and high-grade cod-liver oil. Satisfactory dietary sources are found in butter, cream, cheese, egg-yolk, liver, apricots, carrots, green lettuce, peas, spinach and watercress.

Representative Vitamin A Value of Common Foodstuffs
(Bacharach, 1940)

Vitamin A	β -Carotene
$C_{20}H_{29}OH$	$C_{40}H_{56}$
1 gm. = 2.56×10^6 units.	1 gm. = 1.67×10^6 units.

Daily requirement, as A, 1-3 mg. (2,500-7,500 units).

Daily requirement, as carotene, is probably twice as great.

Source.	A and D Carotene, in Units per gm.	Amount for Daily Requirements.
Milk, cow	0.8-2.5	2 pints-2 gallons
Wheat, entire	2.0-10.0	0.5 lb.-8 lb.
Egg-yolk	2.5-50	0.75 oz.-6.5 lb.
Green leaves	5-100	1 oz.-3 lb.
Butter fat	10-100	1 oz.-2 lb.
Maize, yellow	20-150	0.5 oz.-14 oz.
Liver, beef	75-1,500	1.5 gm.-112 gm.
Palm oil, red	250-3,000	0.5 gm.-28 gm.
Cod-liver oil	600-2,000	1 gm.-12 gm.
Fish-liver oils, various	5,000-50,000	50 mg.-1.5 gm.

As a guide, the chlorophyll content of plants is roughly proportional to the carotene value; and for this reason the green outer leaves of lettuce and cabbage are better foodstuffs than the pale inner leaves.

The vitamin content of animal foodstuffs is primarily determined by the carotene content of the animal's diet. Milk contains both carotene and vitamin A, depending on the species of animal and the time of the year.

Pure crystalline β -carotene has, by definition an A potency of 1.67×10^6 I.U. per gm. The potency of pure A for rat growth has been found by Holmes and Corbett (1937) to be 3.0×10^6 to

3.3×10^6 I.U. per gm., or nearly double that of the carotene, which shows that even under the most favourable conditions, an animal can only form one molecule of vitamin from one of β -carotene. Cattle, sheep, pigs and horses are even less efficient (Brande *et al.*, 1941).

To yield vitamin A in nutrition, a carotinoid must contain at least one β -ionone ring. Substituted rings, such as occur in zeaxanthin and lutein, cannot be utilised. This is a defect of Indian corn (maize), the important animal feeding stuff, as it contains more zeaxanthin than carotene and cryptoxanthin. Carotenes, being highly unsaturated, are easily oxidised, and lose their provitamin A value. Thus, the carotene value of fresh pasture grass is 42–56 mg. per 100 gm. dry material, while that for hay may be only 0.01–0.6 mg.

Tests for Vitamin A.—In addition to the biological tests by feeding, the vitamin may be detected and estimated (1) *spectrometrically*, by observing the extinction coefficient at 328 $m\mu$ in the solution; and (2) *chemically*, by the antimony trichloride colour reaction (p. 204); (3) by measuring the time required for dark-adaptation in order that the subject may recognise a dimly-lit diagram.

Vitamin A₂ occurs in the liver and retina of fresh-water fish. It differs from vitamin A in its ultra-violet spectrum, and in the colour it gives with SbCl₃. Otherwise, both vitamins are closely related, and may occur together in liver oils.

Vitamins D

History.—Contemporary records and paintings show that rickets was prevalent in parts of Europe during the sixteenth century, and in 1650, Glisson published the first description of the disease as a clinical entity.

The concept of an anti-rachitic vitamin is due to Hopkins, who, in 1906, suggested that rickets (or rachitis) is a nutritional deficiency disease. The anti-rachitic factor was believed to be vitamin A, but, in 1922, McCollum and his colleagues found that cod-liver oil, freed from vitamin A by aeration at 120° C., was effective in the cure of rickets. This they correctly attributed to the presence of the factor recognised in 1919 by E. Mellanby as vitamin D.

In 1924 it was announced by Hess, and, independently, the same year by Steenbock, that inert foodstuffs can be rendered anti-rachitic by ultra-violet irradiation. Two years later it was shown that a vitamin precursor occurred in the sterol fraction of the food material, identifiable as ergosterol.

It was thought at first that *calciferol*, the vitamin got by irradiation of ergosterol, was the natural vitamin D, but Bills observed certain differences, and, subsequently, a third form of the anti-rachitic vitamin was obtained from dehydrocholesterol, a naturally occurring sterol. At least seven other forms exist. In current nomenclature, vitamin D is the natural vitamin present in liver oils; vitamin D₂ is calciferol, the irradiation product from ergosterol; and vitamin D₃ is the vitamin obtained by the irradiation or chemical activation of dehydrocholesterol. A fourth form of the vitamin, D₄, has been obtained artificially by irradiation of 22:23-dehydroergosterol and D₅, from 7-dehydrositosterol. Natural D in liver-oils is usually a mixture of D₂ and D₃.

Synthetic *dihydrotachysterol*, or "AT 10" resembles the D vitamins in its hypercalcaemic effect, but it is more effective in promoting urinary phosphate output, and in this respect resembles the parathyroid hormone.

Sources.—Vitamin D is formed by the action of ultra-violet irradiation on certain sterols, and it can occur in Nature wherever this reaction has taken place, the amount depending on the sterol content of the food material and the degree of exposure to sunlight or other source of radiant energy. Vitamin D is associated usually with vitamin A in distribution, and occurs freely in liver oils and other animal fats, including egg-yolk and butter. The provitamin, ergosterol, occurs chiefly in yeast and higher fungi; material containing it is a potential source of vitamin D₂, and may be rendered active by natural or artificial irradiation.

Synthesis of Calciferol.—Ergosterol is exposed to ultra-violet irradiation at 281 mμ until the spectrum shows maximal absorption in that region. Unchanged ergosterol is separated by freezing, and the calciferol is then isolated by dinitro-benzoylation, and recrystallised after hydrolysis of the insoluble dinitro-benzoate. The type-formula for the D vitamins is given on p. 198.

Properties.—Vitamin D₂, or calciferol, C₂₈H₄₄.OH, is isomeric with ergosterol, but differs in optical activity, ultra-violet spectrum, and other properties. The vitamin occurs in colourless needles, m.p. 114.5–117° C., $[\alpha]_D^{20} = +102.5^\circ$, in alcohol. The solutions show an intense absorption band in the region 265 mμ.

Effects of Vitamin D Deficiency.—The vitamin regulates the calcium and phosphate metabolism by promoting phosphate retention, calcium absorption from the intestine, and calcification activity by the osteoblasts of skeletal tissue. Avitaminosis D is characterised by:—

(1) *Subnormal phosphate level in the plasma.*—In man, the in-

organic phosphate value of 2-5 mg. P per 100 ml. may be lowered to 1.5 mg. P.

(2) *Subnormal calcium level in the plasma.*—The total calcium value may fall from its normal level of about 10 mg. per 100 ml. to half this amount.

Values below 7 mg. tend to induce hypocalcaemic tetany unless the fall has been so gradual that the organism has become adapted to the lower level.

(3) *Decrease in the $Ca \times PO_4$ product in plasma.*—Rickets cannot occur if the product Ca (in mg. per 100 ml. plasma) $\times PO_4$ (in mg. P per 100 ml.) is above 40. Severity and rapidity of onset depend on the extent to which the product falls below 36.

(4) *Decrease in the excretion of phosphate and calcium by the kidney.*

(5) *Increase in the excretion of phosphate and calcium by the intestine.*—These conditions are ascribed to defective absorption from the alimentary tract, the contents of which tend to become abnormally alkaline in the absence of vitamin D.

Pathological Conditions Associated with Vitamin D Deficiency

(i) *Rickets.*—This disease, characterised by defective ossification of the growing skeleton, is determined by five aetiological factors: (1) the calcium content of the dietary; (2) the phosphate content of the dietary, including the phosphoproteins; (3) the supply of vitamin D; (4) the degree of exposure to ultra-violet irradiation; (5) the de-calcifying agents in the dietary, the most important of which is *phytic acid*, a constituent of the outer coatings of cereal grain, and present in bran and high-extraction flour. It accounts for the severe form of rickets that follows excessive consumption of cereals, uncompensated by increased administration of vitamin D, Ca^{++} and PO_4^{---} .

(ii.) *Osteomalacia.*—A disease rare in Europe but common among women in certain parts of China. It is an adult form of rickets, and is characterised by de-calcification of the skeleton developing during the later months of pregnancy, when the resources of the mother are being exhausted to supply the foetus.

The domestic proverb, "Every child costs a tooth," is a recognition of this super-tax imposed on maternity.

(iii.) *Dental Caries.*—M. Mellanby (1934) concludes that vitamin D, along with assimilable calcium and phosphate, is necessary for the formation of secondary dentine, and the defence against caries. Although the "average" tooth is of poor structure and liable to decay, its architecture may be improved by proper diet. She advocates a decreased consumption of cereals, or even

their complete removal from the dietary of young children. Vitamin C also appears to be concerned in the maintenance of dental structure.

Vitamin D Standard and Requirements.—The international standard adopted by the League of Nations (1931) is the biological activity of 1 mg. of a standard solution of calciferol in oil. This quantity given daily to a young rat rendered rachitic by a diet free from vitamin D will produce in eight days a characteristic band of calcium deposits in the metaphyses of the long bones. One mg. of crystalline calciferol is equivalent to exactly 40,000 international units. The daily requirement of vitamin D needed to prevent or cure mild rickets in children is about 3,000 units; healthy children and adults, apart from pregnancy conditions, require 200–500 units daily.

Sources of Vitamin D.—Vitamin D, like vitamin A, is stored freely in the liver fats of fishes, the amount varying seasonally, and inversely with the oil content. Expressed in international units per gm. liver oil, representative values are: bluefin tuna, 40,000; swordfish, 10,000; halibut, 2,100; turbot, 260; cod, 100; haddock, 10. The vitamin is also obtainable in egg-yolk, irradiated milk, yeast and other foodstuffs rich in sterols, as well as in the synthetic form, calciferol. Plant products as a class are free from vitamin D, with the exception of the seed fat of the chocolate tree.

Representative Vitamin D Value of Common Foodstuffs

D₂, Calciferol, C₂₈H₄₃.OH

D₃, C₂₇H₄₃.OH.

1 gm. = 40×10^6 units.

Daily requirement for children and for adults, non-pregnant or non-lactating, 5–10 μ g. (200–500 units).

Source.	D in μ g. per gm.	Amount for Daily Requirements.
Milk	0.0001–0.002	2–22 gallons.
Butter fat	0.002–0.05	4 oz.–1 lb.
Egg-yolk	0.04–0.15	1.5 oz.–11 oz.
Cacao butter	0.05	4 oz.–8 oz.
Cacao shell	0.7	7 gm.–14 gm.
Cod-liver oil	1–6	1 gm.–14 gm.
Fish-liver oils, various	25–6,000	1 mg.–400 mg.

An intrinsic source of the vitamin is provided by the direct insolation or artificial irradiation of the human subject.

Hypervitaminosis D.—Vitamin D is the only known vitamin capable of causing pathological disturbances when given in very large amounts. These disturbances include increased calcification of bone and abnormal calcification of soft tissues; the immediate cause, according to Harris, is the hypercalcaemia and hyperphosphataemia produced by the vitamin. Hypervitaminosis D is improbable in human nutrition, the quantities of the vitamin ingested in the diet, or administered therapeutically are usually far below the danger level.

Vitamin D deficiency is a serious menace to children living in northern latitudes, and especially dwellers in crowded areas, where the sunlight available in the winter months is insufficient to synthesise the vitamin from body sterols.

Tests for Vitamin D.—*Biological* tests include calcification of the bones of young animals as shown by (i.) radiography of the distal ends of the ulna and radius; by (ii.) the "line test," in which the bone is sectioned and stained with silver nitrate; and by (iii.) analysis of bone ash for Ca and PO_4 . Spectrographically, the vitamin may be assayed by means of its ultra-violet absorption band.

Vitamins E

The Tocopherols, α -, β - and γ -, occur as anti-sterility factors, first recognised by Evans (1922), and by Sure (1923), who observed, independently, that rats reared on purified diets supplemented by vitamins displayed subnormal fertility in the second generation. Fertility could be restored by addition of various natural food-stuffs, notably lettuce, meat, wheat-germ, rolled oats, or milk fat in large amounts.

Sherman has pointed out that the description of vitamin E as the anti-sterility vitamin is misleading, for although it is essential for reproduction it is not any more essential than vitamin A.

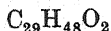
Vitamin A deficiency interferes with ovulation, whereas vitamin E deficiency interferes with placental function in the female, and with germ-cell maturation in the male. E has an absorption band about 298 $\text{m}\mu$. In its natural sources it is accompanied by at least two related compounds with absorption bands at 267 $\text{m}\mu$ and 294 $\text{m}\mu$. The name α -tocopherol (*tokos*, child-birth, and *phero*, to bear) has been given to the active alcohol, 3 mg. of which administered daily restores fertility to rats on a vitamin E deficient diet.

Vitamin E Standard.—The international unit is the activity of 1 mg. synthetic racemic α -tocopherol acetate, $C_{31}H_{52}O_3$, which is the average amount that, when given by mouth daily, prevents resorption gestation in a pregnant rat on an E-free diet. β - and γ -tocopherol have half the potency of the α -form.

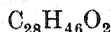
Sources.—A rich source is the unsaponifiable fraction of wheat-germ oil; other good natural sources are lettuce, watercress, and wheat preparations that include intact germ; moderately good sources are entire cereals, molasses, cotton-seed oil and hemp-seed oil. Animal foodstuffs are poor in the vitamin, and it is absent from liver oils of fish.

Representative Vitamin E Values of Foodstuffs

α -Tocopherol



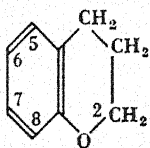
β -Tocopherol



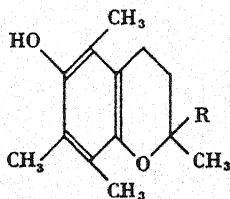
Daily requirements for 150 days during pregnancy, 10–50 mg. Requirements for males and non-pregnant females, unknown.

Source.	E in mg. per gm.	Amount for Special Requirement.
Wheat, entire . . .	0.002–0.005	4 lb.–60 lb.
Lettuce leaf, fresh . . .	0.02–0.1	0.25 lb–6 lb.
Cotton-seed oil . . .	0.1–0.5	1 oz.–20 oz.
Wheat germ . . .	0.15–0.8	0.5 oz.–10 oz.
Wheat germ oil . . .	2–10	1 gm.–25 gm.

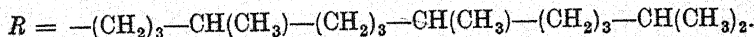
Structure of Vitamin E.—All are derivatives of a double-ring nucleus, *chromane*, in which a methyl group and a C_{16} side chain, are attached at position 2, an hydroxyl group at 6, and a methyl group at 8. In α -tocopherol, there are also a methyl group at 5 and at 7; in β -tocopherol, the group at 7 is absent, while in γ -tocopherol, the group at 5 is absent.



Chromane.



α -Tocopherol.



Effects of Vitamin E Deficiency.—Neither oestrus cycle nor implantation of ovum is affected, but foetal death occurs at an early stage in development owing to defects in the allantois and yolk sac.

In the human subject, avitaminosis E is a factor in habitual abortion, sterility, both male and female, and excessive hæmorrhage after parturition, though this last may be due to lack of vitamin K. Vitamin E is necessary in muscle metabolism, and this may be its primary function (Pappenheimer, 1943).

Avitaminosis E is characterised by degeneration of uterine and skeletal muscle, as can be traced by the increase in urinary creatine. The vitamin has been advocated for the treatment of muscular dystrophy and various neuro-muscular disorders.

Tests for Vitamin E.—*Biological* tests are based on the ability of the vitamin to increase the fertility percentage of rats on an E-free diet. *Spectroscopic* tests depend on measurement of the absorption band about $265\text{ m}\mu$ of the oxidised vitamin (John, 1939). *Chemical* tests depend on the formation of a red colour when the vitamin is warmed with HNO_3 in alcoholic solution (L. Smith, 1939).

Vitamins K

In 1934, Dam and Schönheyder described a deficiency disease in chicks, characterised by anæmia, increased blood-coagulation time, pathological changes in the gizzard, and tendency to hæmorrhage. The condition was traced to the lack of a specific fat-soluble, alkali-labile but thermostable factor, vitamin K.

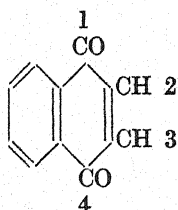
The vitamin was isolated, in 1939, by Dam, Karrer *et al.*, and a related vitamin, K_2 , was isolated by Doisy *et al.*

Sources.—Vitamin K occurs in hog-liver fat, and to a lesser extent in the liver fat of the dog and the cod. Its principal source is the lipid fraction of green vegetables. The vitamin is a yellow oil, soluble in fat solvents and thermostable, but destroyed by alkalis. The unit is defined as the smallest dose, per gm. body weight, which given for three days is sufficient to restore the blood-coagulation time to normal in experimental animals.

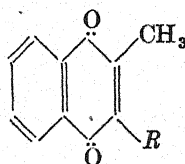
Vitamin K_2 occurs in most bacteria, including those present in the intestine, which may provide some of the vitamin needs of the animal. K is necessary for production of *prothrombin*, a factor in blood coagulation, made in the liver and circulated in the plasma. Both vitamins are effective when given by mouth, but require bile acids for their absorption. Hæmorrhages due to failure of clotting may occur in obstructive jaundice, chronic intestinal

disorders, and bile-duct tumours, where the absorption of K is restricted. It also arises from prothrombin deficiency in both mother and child, at parturition. Hence, green vegetables, such as spinach, tomatoes, or other good sources of K should be given to mothers during the last months of pregnancy, and K should be given to children during the critical first three days after birth.

Structure of Vitamins K.—Both are derivatives of 1:4-naphthoquinone, in which there is a methyl group at position 2, and either a *phytyl* group (K_1) or a *difarnesyl* group (K_2) at position 3. Unlike vitamins E, the long-side chain is not necessary for activity, and can be effectively replaced by H, as in the synthetic K vitamin, 2-methyl-1:2-naphthoquinone, which is three times as potent as K_1 .



1:4-Naphthoquinone.



Vitamin K.

K_1 , $R = -CH_2 \cdot CH : C(CH_2)_7 - (CH_2)_4 - C(CH_3)_2 - (CH_2)_7 - CH_3$.
 K_2 , $R = -CH_2 \cdot [CH = C(CH_3) \cdot CH_2 \cdot CH_2]_5 - CH = CH \cdot (CH_2)_2 \cdot$
 2-Methyl-1:4-Naphthoquinone, $R = H$.

Tests for Vitamin K.—Biological tests are based on the reduction of the blood-coagulation time in 15-day chicks on a vK-free diet, or on the estimation of the actual amount of prothrombin present in the blood of various animals (Quick, 1940). Chemical tests include the measurement of the blue colour formed when vK in alcoholic solution is mixed with sodium diethyl-dithiocarbamate and alcoholic sodium hydroxide (Irreverre and Sullivan, 1941).

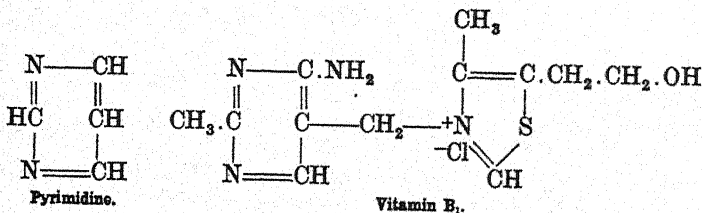
THE WATER-SOLUBLE VITAMINS

The Vitamin B Complex.—Recognition of a group of water-soluble vitamins dates from 1911, when Osborne and Mendel reported the presence of one "growth determinant" in milk free from protein and fat, and another in separated milk fat. McCollum and Davis also reported the presence of two distinct growth factors in natural foodstuffs; one of which they called "water-soluble B" to distinguish it from the other factor, "fat-soluble A." Later on they showed that water-soluble B was curative of beri-beri, and they concluded it was identical with the "vitamine" present in rice husks, and shown to have anti-neuritic properties.

Resolution of Vitamins B.—The complex can be resolved into a thermo-labile, anti-neuritic factor, B_1 , and various thermo-stable "biotic factors," including B_2 , B_6 , nicotinic and pantothenic acid, biotin, inositol, etc.

All the members of the B complex are present in ordinary yeast, and might be described as the "yeast vitamins."

(1) **Vitamin B_1 .**—*Aneurin*, or thiamine, $C_{12}H_{17}ON_4SCl$, formerly termed "vitamin F," the anti-neuritic member of the B-complex, has been isolated in crystalline form, m.p. 250° , from rice bran, in 1926, by Jansen and Donath, and from yeast, in 1931, by Windaus *et al.* The structure was elucidated, in 1936, independently, by Williams, and by Grene. The vitamin is a thiazol derivative of 2-methyl-6-amino-pyrimidine, and occurs naturally in the form of a chloride. It is extracted by water or alcohol, an acidified 50 per cent. mixture being the most effective, and may be precipitated by alkaloidal reagents, such as picric acid, and purified by adsorption by charcoal or Fuller's earth. It is stable in neutral or acid solution, but is rapidly inactivated by alkalis at the temperature of boiling water. On oxidation it yields a yellow pigment with a blue fluorescence, *thiochrome*, formerly isolated from yeast, and synthesised by Todd, Bergel and their colleagues (1936).



Sources.—The most potent sources of vitamin B_1 are concentrates prepared from yeast, wheat-germ or rice-polishings. Satisfactory food sources are entire cereals (the vitamin is located chiefly in the germ and the bran), nuts, leguminous seeds and malt extract. Fresh vegetables, fruits and animal products, such as cheese, eggs, fish roe, kidney, liver and milk, are moderately rich in the vitamin.

Functions.—Thiamine pyrophosphate, or *co-carboxylase*, is necessary for transfer of CO_2 in the various carboxylation-decarboxylation reactions in carbohydrate metabolism, and, consequently, the vitamin is present in all living organisms. In the absence of the vitamin, pyruvic acid accumulates, and induces the characteristic signs of avitaminosis B_1 .

Effects of Vitamin B_1 Deficiency.—(i.) *Beri-beri*.—This disease is endemic among the rice-eating communities of the East, including India, the Malay Peninsula and Japan, where the diet is almost

entirely restricted to rice that has been "polished" by removal of the outer husk to improve storage qualities. Beri-beri also appears among wheat-eating communities, such as those of Labrador and Newfoundland, who subsist mainly on refined wheat flour. Infantile beri-beri is a direct consequence of a vitamin deficiency in the diet of the nursing mother, and is responsible for a large part of the enormous infantile mortality of India and the East in general. Beri-beri is a polyneuritis characterised by loss of muscular co-ordination, gradual paralysis of the limbs, alimentary disturbances (indigestion, constipation and colitis), degeneration of the heart muscle, and general emaciation, often associated with dropsy.

Eggleton (1939) believes that zinc deficiency is a factor in evoking beri-beri. Zn is a constituent of the enzyme, *carbonic anhydrase*, concerned in the release of CO_2 from H_2O , and may be essential for all carboxylation systems.

(ii.) *Avian Polyneuritis*.—When birds are fed on an exclusive diet of polished rice and water no ill effects are seen for several weeks. Then signs of acute polyneuritis appear rapidly. The bird is unable to fly, to walk, and, eventually, to stand. Exhaustion and death follow in a few days unless the vitamin be supplied. Recovery is dramatic.

The key observation on the association of polished rice with beri-beri and avian polyneuritis was made by the Dutch physician, Eijkman, when in Java, in 1897. He noticed that domestic fowl fed on the polished rice developed the same type of neuritic disorder as that found among prison inmates on the same dietary, whereas birds fed on unpolished rice were free from the disease. Grijns (1901), his colleague, concluded that the disease was due to a deficiency, a negative factor, and not a positive infection or toxin. Similar and independent conclusions were reached by Frazer and Stanton, working in the Malay Peninsula (1909), and by Chamberlain, in the Philippine Islands (1910). Twenty years later, Peters observed that oxidation of pyruvate and lactate is subnormal in brain tissue from vitamin B_1 deficient pigeons, and thus got the clue to the significance of B_1 in metabolism.

(iii.) *Bradycardia*.—This is a slowing of the heart-beat owing to sinus changes, and responds rapidly to vitamin therapy. It is a feature of rat beri-beri. The phenomenon has been used by Birch and Harris (1934) for the electrocardiographic assay of vitamin preparations. Rats that have been depleted of vitamin B_1 are fed, under controlled conditions, with graded doses of the test materials, and the resulting cardiac response is observed. In infants and human adults, a tachycardia, or increased heart rate, is evoked by B_1 deficiency (Drury, Harris and Maudsley, 1930).

(iv.) *Gastro-intestinal Dysfunction*.—Impairment of appetite, decreased motility of the stomach and loss of tonus, subnormal temperature, anhydræmia, and decreased resistance to fatigue go

to make up a syndrome arising from disturbed innervation of the alimentary tract.

(v.) *Pyruvate Accumulation*.—The pyruvate content of blood, urine, and cerebro-spinal fluid rises characteristically in beri-beri, and is specifically reduced by injection of B_1 .

Vitamin B_1 Standard and Requirements.—The 1938 International Unit is 3 μ g. of crystalline thiamine chloride hydrochloride. Sherman's unit is the amount of vitamin required daily to enable a growing rat to gain in body weight by 3 gm. a week over an experimental period of four to eight weeks. It is about two-thirds of the international unit.

Vitamin B_1 Value of Common Foodstuffs
(Expressed in International Units per gram)

Material.	Vitamin B_1 .	Material.	Vitamin B_1 .
Beef, raw . . .	0.5	Fig, dried . . .	1.0
Kidney, sheep . . .	0.9	Nuts	0.8-2.0
pig	3.4	Wheat, entire . . .	2.3-3.4
Liver, ox	1.5	germ	5.9-18.7
Fish, raw	0.3	Flour, 85 per cent.	
Milk	0.2	extraction . . .	0.9
Cheese	0.0-0.2	75 per cent.	
Egg-yolk, boiled . .	1.4	extraction . . .	0.45
white, "	a trace	Oatmeal, dry . . .	3.25
Beans	1.2-1.6	Rice, polished . . .	0.0
Carrot, raw	0.6	bran	5.6-7.6
Lentil, uncooked . .	2.1	Coffee bean, ground	1.4
Lettuce	0.9	Cocoa	0
Potato, boiled . . .	0.3	Brewer's yeast, dried	6-23.0
Fruit, fresh	0.3-1.0		

Rich sources of the vitamin are the commercial preparations *Atuzyme*, *Marmite*, *Gye*, and *Bemax*, and various malt extracts.

The adult human requirements have been assessed at 350-1000 international units, *per diem*, which represents 1 to 3 mg. of the hydrochloride of thiamine chloride, $C_{12}H_{17}N_4SOCl$, HCl .

Vitamin B_1 in Plant Growth.—Thiamine is essential in plant metabolism. Some green plants and some fungi are autotrophic, in that they synthesise sufficient for their complete requirement. Others are heterotrophic in regard to thiamine, and are represented by many parasitic types, bacteria, yeasts and fungi, the growth of which requires an external supply of the vitamin. R. Williams regards the universal necessity for thiamine as proof of a common chemical ancestry of all forms of life.

The vitamin B_1 need depends, chiefly, on the amount of glucose metabolised, each gram of which requires 0.8 units of B_1 .

A normally balanced diet should include 0.5 mg. (167 I.U.) B_1 , per 1,000 kilocalories intake. A diet rich in carbohydrates requires 0.6 mg. (200 I.U.) B_1 , per 1,000 kilocalories.

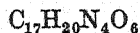
Tests for Vitamin B_1 .—Biological tests include (i.) the cure of retracted neck in pigeons after a twenty to thirty days' diet of polished rice and water; (ii.) the cure of convulsive paralysis and (iii.) bradycardia in rats on a deficiency diet; (iv.) restoration of normal growth rate (weight increase) to young rats on a B_1 -free diet; stimulation of growth of yeasts and moulds.

Chemical tests include (i.) oxidation by alkaline ferricyanide to thiochrome (p. 412), the concentration of which can be measured from its fluorescence in ultra-violet light; (ii.) various colour reactions (Rosenberg, 1942).

(2) **Vitamin B_2 , Riboflavin.**—The residual B_2 -complex has been resolved into riboflavin and an associate sub-group of B vitamins. Riboflavin is one of the lyochromes, or water-soluble yellow pigments that impart a greenish-blue fluorescence to various plant and animal extracts. Riboflavin has been obtained in crystalline form from egg albumin, yeast, liver, kidney and urine. Its structure and chemical relationships are given on p. 226. Both natural and synthetic riboflavin have the growth-promoting properties characteristic of vitamin B_2 preparations, but are devoid of anti-dermatitic and pellagra-preventing properties possessed by other members of the sub-group.

Sources.—The chief sources of vitamin B_2 complex are concentrates from yeast and from whey. Important food sources are: yeast, animal organs, including liver, kidney and spleen, lean meat, egg-white, milk, and some vegetables, such as beet and turnip greens, spinach, potato and watercress.

B_2 , Riboflavin



Daily requirement, 1 mg.—3 mg.

Source.	B_2 in mg. per gm.	Amount for Total Requirement.
Wheat, entire	0.3	7.5 lb.—22.5 lb.
Cabbage	0.5	4.5 lb.—13.5 lb.
Fish-liver	0.5–10	4 oz.—13.5 lb.
Meat	1–4	9 oz.—6 lb.
Maize	1.5–2	1 lb.—4.5 lb.
Eggs	4–6	6 oz.—1.75 lb.
Yeast, dried	20–40	1 oz.—6 oz.
Liver and kidney . .	20–40	1 oz.—6 oz.

B₂ is not hard to obtain from an average mixed diet containing meat, milk and vegetables. Thus, 1 pint of milk or 3 oz. of liver can be expected to supply the daily requirement.

Effects of B₂ Deficiency.—*Ariboflavinosis*, due to lack of B₂, according to Sebrell (1941) is shown by (1) pallor and fissure of the *macosa* in the angle of the lip, the condition being termed *cheilosis*; (2) abnormal redness of lips and tongue; (3) scaly desquamation about nose and ears; (4) ocular lesions, including capillary invasion of cornea, soreness of the eyes, and hypersensitivity to light. *Cheilosis* has been cured by 2–6 mg. doses of riboflavin, but some cases respond better to nicotinic amide or to pyridoxin, the functions of which may overlap.

Functions.—Riboflavin nucleotides, when combined with proteins, form the flavoprotein enzymes, which include several important dehydrogenases. Thus, the function of B₂, like B₁, is to provide a co-enzyme necessary in tissue metabolism. The human requirement for normal health is estimated to be 40 to 50 μ g. of riboflavin per 100 kilocalories of food (Sherman and Lanford, 1938; Bessey, 1938).

Tests for Vitamin B₂.—Biologically, riboflavin can be assayed by its effects on the growth of young rats on a B₂-free diet, and V. Euler (1938) has proposed as an internal unit the potency of 5 mg. of crystalline riboflavin, which amount evokes a weight increase of 0.8–1.0 gm., daily, in young rats. B₂ can also be estimated by the intensity of its fluorescence, which shows a maximum at 565 m μ in solutions of pH 6.

Vitamin B Refection.—Spontaneous recovery from avitaminosis B₁ + B₂ while on a deficiency diet has been observed, especially among rats, and is accompanied by a marked change in the bacterial flora of the intestine. It is due to local synthesis of the vitamins by micro-organisms in the lower alimentary tract, and is termed *refection*.

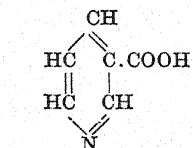
Biotic Factors.—Members of the B complex, in addition to providing special vitamin requirements of higher animals, are essential for growth of micro-organisms. Robinson (1944) uses the term *biotic* to include all such specific compounds of natural origin, synthesised by micro-organisms, and required by micro- and higher organisms.

(3) **Vitamin B₆, Pyridoxin.**—A dermatitis occurs in rats fed on experimental diets, and somewhat resembles human pellagra. It is cured specifically by a factor which accompanies the B complex in yeast, cereal germ and bran, muscle, liver and milk. According to Lepkovsky (1939–42), it is necessary for hæmoglobin construction. Pyridoxin, or *adermine*, as it is also termed, is 1-methyl-2-

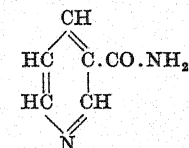
hydroxy-3:4-dihydroxy pyridine. Pyridoxyl phosphate is the co-enzyme of tyrosine decarboxylase.

(4) **The Pellagra-preventing Factor.**—Vitamin B sub-group was defined as "the more heat-stable, water-soluble dietary factor necessary for growth and health and prevention of characteristic skin lesions," among which was often included pellagra.

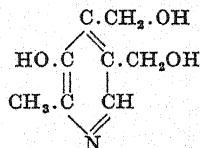
Pellagra, or "rough skin," is a disease common in Rumania, South Russia, the southern States of America, and in Italy. Described by Aykroyd as "perhaps the most horrible of all food deficiency diseases," it is characterised by dermatitis, pigmentation and thickening of the skin, inflammation of the tongue and intestinal tract, and nervous disorders leading to atrophic paralysis and dementia. Some of the signs resemble the condition of "black tongue" found in dogs on a diet lacking vitamin B complex. The pioneer work of Goldberger (1910-30) showed that endemic pellagra was a poverty disease and could be abolished by the use of fresh meat and green vegetables, which he found to contain a pellagra-preventing, or p.p. factor, now identified as nicotinic acid (Spies, *et al.*; Elvehjem, *et al.*, 1937). Nicotinic acid was isolated by Funk in 1911 from rice bran, but its significance was overlooked. Nicotinic acid is β -carboxy pyridine, and is a constituent of the alkaloids nicotine and trigonelline. Its amide is a constituent of the co-enzymes co-dehydrogenase I and II, which accounts for the importance of nicotinic acid in animal nutrition. The effective daily dose in the cure of human pellagra is 0.5 gm.



Nicotinic acid
(β -carboxy pyridine).



Nicotinic amide
(p.p. factor).



Pyridoxin
(vitamin B₆).

Nicotinic Acid, or "Niacin," C₅H₅N(COOH).

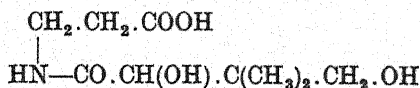
Daily requirement, 5-10 mg.

Source.	Nicotinic Acid, mg. per gm.	Amount for Daily Requirement.
Milk, cow's	10	20 oz.-40 oz.
Meat, beef or pork	40-60	3 oz.-8 oz.
Wheat, entire	50-60	3 oz.-7 oz.
Kidney	50-60	2.5 oz.-7 oz.
Liver	125	0.75 oz.-1.5 oz.
Yeast, dried	550-650	0.25 oz.-0.75 oz.

While nicotinic amide may be regarded as the actual vitamin, nicotinic acid, the provitamin, is equally effective, but less suitable for clinical administration, since it has an unpleasant vasodilator effect not shown by nicotinic amide in moderate or large doses.

The only good sources of nicotinic acid or amide in the diet are yeast, animal tissues, and bread from high extraction (80–85 per cent.) flour. *Nicotine*, α -N-methyl-pyrrolidine- β -pyridine, the poisonous liquid alkaloid of the tobacco plant, yields nicotinic acid on oxidation, but the human body is unable to effect this desirable decomposition. Nicotinic acid is excreted in the urine as a methylation product, *trigonellin*.

(5) **Pantothenic Acid.**—In 1933, Williams and his associates showed that liver extracts contained a factor capable of promoting growth of yeasts and bacteria, and similar in nature to one of the components of the *bios* complex got from yeast and bran (p. 509). The factor was also found to be capable of curing a nutritional dermatitis in chicks (Jukes, 1939; Woolley *et al.*, 1939), and was eventually isolated, identified as a peptide of β -alanine and α : γ -dihydroxy- β : β -dimethyl propionic acid, and synthesised by Williams in co-operation with the Merck laboratories (1940).



Pantothenic Acid.

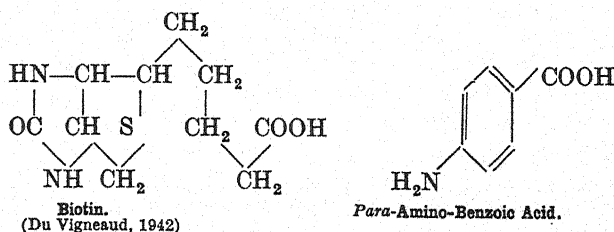
The name indicates the almost universal distribution of the vitamin in both plants and animals. Its significance in human nutrition is not yet known, but it is necessary for normal growth of young rats and mice, and for prevention of a symmetrical depigmentation, or nutritional *achromotrichia*, in which the fur turns grey. The amino acid, β -alanine, is only known to occur in pantothenic acid, anserine and carnosine.

(6) **Folic Acid.**— $\text{C}_{15}\text{H}_{15}\text{O}_8\text{N}_5$ (Mitchell and Williams, 1944), a growth-factor for rats, chicks, and other micro-organisms. It has also got anti-anæmic properties. Originally obtained from leaves, it occurs in liver, kidney, and yeast, and appears to be a xanthopterin (p. 228).

(7) **Biotin, Vitamin H.**—In 1916, Bateman observed that toxic disturbances can arise from an excess of raw egg-white in the diet

of the rat. This was confirmed, in 1927, by Boas, who found that the condition could be cured by a factor in vegetables, termed *vitamin H*, and shown later by Györgyi, Du Vigneaud and others (1941) to be identical with *biotin*, a component of the *Bios* complex (p. 509). Biotin is found chiefly in yeasts, pollen and seed, and is a micro-constituent of animal tissues. The toxic factor in egg-white has been isolated as a crystalline protein, *avidin*, which has the power of combining with biotin and rendering it unavailable for nutrition. On cooking the egg, this avidin effect is abolished.

Biotin deficiency is shown by: (1) *dermatitis*. In man, extreme pallor of the skin and mucous membranes is followed by a dry dermatitis. A similar dermatitis is found in chicks and in rats, which also show a characteristic "spectacle effect" zone round the eyes. (2) Loss of appetite (*anorexia*), lassitude and somnolence, rapidly relieved by injection of biotin. The daily human requirement is provisionally assumed to be 0.1 mg., the need being much greater if raw egg-white be included in the diet.



Tests.—Biotin is assayed by its curative effect on chicks or rats on a diet in which raw egg-white is the only protein. Other tests depend on the power of biotin to promote the growth of yeast and bacteria.

(7) **Para-Amino-Benzoic Acid.**—The ability of pantothenic acid to protect against depigmentation of the hair has been disputed by Williams and by Elvehjem, and Ansbacher (1941) claims that the effective agent is *para*-amino-benzoate, which is widely distributed in plants and animals; yeast being particularly rich, with a concentration of about 0.8 mg. per 100 gm. The vitamin is a component of the *Bios* complex, and seems to be necessary for the growth of all micro-organisms. Sieve (1941) claims that a daily dosage of 100–200 mg. restores the pigmentation of human hair. The vitamin can protect bacteria from the effects of sulphonamide drugs, which, conversely, can be regarded as "vitamin inhibitors."

(8) **Inositol.**—This important component of the *Bios* complex (p. 509), has a physiological action in higher animals, shown by increased motility of the stomach and small intestine (Martin *et al.*,

1941), and by prevention of cholesterol accumulation in the liver (Gavin and McHenry, 1941). Inositol-deficiency is also said to retard the growth of rats, and evoke a dermatitis with depigmentation of the fur and "spectacle effect." The vitamin, possibly, may operate indirectly by promoting the growth of micro-organisms in the alimentary tract, and thus supplying the animal's need by locally made biotics of the B group.

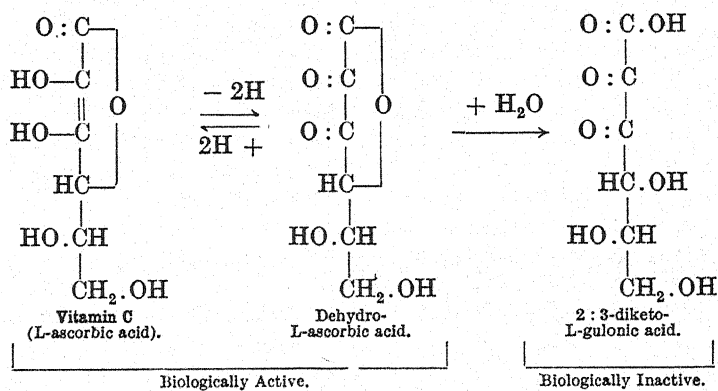
Vitamin C

Vitamin C, Ascorbic Acid.—The anti-scorbutic properties of fresh vegetables and citrus fruits (lemon, lime and orange) were known to the Dutch sailors in the fifteenth century, and during the reorganisation of the British Navy by Jervis and Nelson, lime juice was included in the service dietary. The remedy proved of some value at a time when disease was more dangerous than gun-fire, but even as late as 1916 it was believed by many that scurvy arose from infection. Between 1907 and 1912, Holst and Frölich produced experimental scurvy in guinea pigs by keeping them for two to three weeks on a diet of oats and bran, and showed that the condition resembled human scurvy in all respects, and, like it, was cured promptly by fresh fruits or vegetables. The anti-scorbutic material was fractionated by Bezssonoff, King, Zilva, and other workers, who found that it was associated with the power of decolourising the indicator dye, 2:6-dichlorophenol indophenol, and Tillmans, in 1932, suggested that the vitamin was the actual reducing substance. Vitamin C was obtained from lemon juice by Waugh and King in 1931 and proved to be identical with *hexuronic acid*, $C_6H_8O_6$, isolated, in 1928, from adrenal cortex by Szent-Györgyi, who later recognised its anti-scorbutic properties. The structure was elucidated by Haworth, and confirmed by two independent syntheses, in 1933 (Hirst and Haworth, Reichstein), and the vitamin was renamed *ascorbic acid*.

Properties.—Ascorbic acid, $C_6H_8O_6$, m.p. 192° , $[\alpha]_D = +24^\circ$, is a colourless compound, very soluble in water, and possessing strong reducing properties. The cold solution is able to bleach indicator dyes, reduce Fehling's reagent, and blacken silver nitrate. This last reaction has led to the suggestion that ascorbic acid is responsible, in part at least, for the local silver staining observed in the "argentophil" inclusions present in many cells. Ascorbic acid is the most unstable of all the vitamins. It readily undergoes reversible oxidation followed by hydrolytic destruction, especially in warm, alkaline solutions, and in presence of traces of Cu.

Fresh fruits and vegetables have been shown by Zilva to contain an enzyme, *ascorbic oxidase*, which contributes to the disappearance of the vitamin when plant products are cooked or preserved. Ascorbic acid can be protected by avoiding excessive maceration of the material and contact with copper during processing.

On oxidation, ascorbic acid loses two hydrogen atoms and is converted into *dehydro-ascorbic acid*, which is still biologically active, and can be reconverted into the vitamin by reducing agents, such as H_2S . In neutral or alkaline solution, dehydro-ascorbic acid is spontaneously hydrolysed to 2:3-diketo-L-gulonic acid, a derivative of the sugar L-gulose. This acid has no anti-scorbutic properties, and cannot be reconverted into the vitamin by reduction. Since the vitamin is derived from the L-sugar, it is termed L-ascorbic acid. Its laevorotary isomer, D-ascorbic acid has been synthesised and shown to be biologically inactive.



Both forms of ascorbic acid are lactone derivatives of the substituted gulonic acid. When the lactone ring had been opened by hydrolysis, the vitamin is irreversibly inactivated, and readily oxidises to oxalic and L-threonic acid.

Sources.—The synthetic vitamin is obtainable commercially. Other highly potent sources are concentrates prepared from black-currents and rose hips. Natural food sources are represented by citrus fruits and raw vegetables, especially tomato, celery, onion and rhubarb. The best animal sources are liver and fresh milk.

Hopkins (1938) has shown that lactoflavin in presence of light catalyses the oxidation of ascorbic acid, and thus is responsible for its spontaneous destruction in milk.

Root vegetables are poor in C; the potato, however, is important, because of the quantities eaten, which in some European communities was 1-2 lb. per person daily.

The vitamin is present in almost every part of higher plants and animals, being maximal in glandular tissue, and minimal in muscle and storage fat. It is formed rapidly in germinating seeds, which provide a valuable emergency source of the vitamin.

Functions.—The reversible oxidisability of the vitamin suggests that it can act as a hydrogen-transport agent in cell metabolism, and since its formation precedes that of chlorophyll and the carotinoids in the growing plant, it is probably concerned in the mechanism for synthesising these pigments.

In animals, vitamin C is necessary for the formation of collagen fibres, bone tissue regeneration, and wound repair. Lewine (1941) reports that abnormal products of tyrosine appear in the urine of infants suffering from scurvy, which suggests that the vitamin may function in some aspects of protein metabolism. Many of the features of scurvy may be attributed to defective production of protein material by the mesenchymal tissues.

Effects of Vitamin C Deficiency.—(i.) *Infantile Scurvy.*—This condition is found in children fed exclusively on sterilised or artificial dietaries, or by mothers whose milk is deficient in ascorbic acid. After a period of anæmia and irregular growth, the characteristic syndrome appears: sore gums, periosteal hæmorrhages at the joints, subcutaneous hæmorrhages and hæmaturia.

(ii.) *Adult Scurvy.*—This is seen among explorers and others on dietaries deprived of fresh foodstuffs for prolonged periods, and is always a potential danger in communities living on artificially modified foods, especially when individual requirements are increased by pregnancy, lactation or disease. The scorbutic syndrome includes hæmorrhages from mucous membranes, skin, joints, limbs and bone marrow, spongy and bleeding gums, pain and swelling in joints and limbs. The disease progresses to complete incapacitation, and terminates fatally.

Many of the signs of scurvy are referable to increased capillary permeability and escape of blood into the tissues, owing to lack of vitamin P.

(iii.) *Dental Disorders.*—Spongy and bleeding gums, decay and loosening of the teeth, pyorrhœa: all have been attributed to avitaminosis C, and have been cured or checked by ascorbic acid. The vitamin appears to be necessary for maintaining the activity of the formative cells, odontoblasts, ameloblasts and osteoblasts.

(iv.) *Delayed Wound Repair.*—In scorbutic subjects, the fibroblasts that normally invade zones of injury are less active, and scar tissue may fail to develop.

The natural distribution of vitamins C and P is almost identical,

hence, unless artificially induced, scurvy is always a mixed deficiency disease.

Vitamin C Standard and Requirements.—The 1934 International Standard is the biological activity of 0.05 mg. of pure L-ascorbic acid, which now replaces the former standard, 0.1 ml. of fresh lemon juice. Sherman's unit is the daily amount of vitamin just sufficient to protect a standard guinea pig from the effects of a scorbutic diet. Sherman's unit is approximately equal to 10 international units. The minimal daily requirement to prevent any possible prescorbutic condition in man is about 19–25 mg. ascorbic acid, the advisable intake for a 70 kg. subject being about 60 mg., or nearly 1 mg. per kg. body weight. The requirements of a child are about twice as much. Many animals, notably the rat, are immune from scurvy, and presumably can synthesise the vitamin, or obtain it from their intestinal flora. Man, the monkey, and the guinea pig, are entirely dependent on exogenous sources.

On an ordinary mixed diet, 25–30 mg. of ascorbic acid are excreted daily in the urine. The animal organism is unable to store the vitamin beyond a tissue saturation limit, which is, generally, less than 0.5 mg. per gram. Blood plasma ascorbic acid ranges from 0.8 to 2.4 mg. per 100 ml., the average being 1.6. In scurvy the plasma has a value of 0.4, or less.

Vitamin C Value of Common Foodstuffs

Vitamin C Values

Ascorbic Acid $C_6H_8O_6$

Daily requirement, 25–75 mg. (500–1,500 units).

Source.	C in mg. per gm.	Amount for Daily Requirement.
Apple	0.001–0.2	4 oz.–1.5 cwt.
Milk, cow's	0.01–0.03	1.5 pints–12 pints
Banana	0.01–0.15	5 oz.–17 lb.
Potato, raw	0.1–0.4	2 oz.–2 lb.
Liver	0.1–0.8	1 oz.–1 lb.
Citrus juice	0.2–0.8	0.5 oz.–14 oz.
Green leaves	0.2–1.25	0.5 oz.–14 oz.
Adrenal, ox	0.8–2.0	0.5 oz.–2 oz.
Black currant juice	1.5–2.5	0.5 oz.–2 oz.

Additional sources, expressed in mg. C. yielded by 1 gm., are :—

Material.	Ascorbic Acid.	Material.	Ascorbic Acid.
Orange juice .	0.22-0.89	Milk, human .	0.10-0.15
Lemon juice .	0.47-0.73	Spinach, raw .	1.2-4.8
Tangerine juice .	0.10-0.78	boiled .	0.2-0.3
Marmalade .	0.06-0.14	Cabbage, raw .	0.8-1.4
Tomato juice .	0.17-0.7	boiled .	0.01-0.09
Apple juice .	0.02-0.1	Rose hips .	3-8
Grape-fruit juice .	0.59-0.65	Walnut shell, green .	9-12
Strawberry .	0.17-0.6		

Bacharach and his colleagues, from whose work many of the above data have been taken, report that storage for a month causes a 20 per cent. loss of ascorbic acid in oranges, and a 6 per cent. loss in lemons. The loss, however, was negligible in juices properly concentrated and stored. The vitamin C value of cooked vegetables depends largely on the time and condition of the cooking. Where sodium bicarbonate is added to improve the colour, and the boiling is prolonged, the ascorbic value drops to zero. Rapid and brief sterilisation of milk by heating to about 90° (pasteurisation) has little effect on the vitamin, boiling for more than ten minutes may complete the inactivation that has been in progress ever since the milk was secreted.

Estimation of Ascorbic Acid.—This may be done spectrometrically, from the extinction coefficient of the characteristic absorption band at 245 m μ , or by titration with a suitable oxidation indicator.

Many animal tissues contain reducing substances, notably adrenaline, glutathione, and cysteine, and the specificity of the reduction method for estimating vitamin C was uncertain until it was shown that the redox indicator 2 : 6 dibromophenol-indophenol is not affected in acid solution by adrenaline or glutathione, although it is rapidly reduced by ascorbic acid. Furthermore, mercuric acetate does not precipitate ascorbic acid, although it removes other reducing factors, including glutathione and cysteine. By adaptations of these methods, Harris and other workers have been able to estimate the ascorbic acid content of various substances and to show that it parallels the vitamin C content, as determined biologically.

Oxidised ascorbic acid does not reduce the indicator, so the mixture is treated first with H₂S, which both reduces the dehydro-ascorbic acid, and removes any excess of mercury that may remain from the preliminary purification.

On addition of a few drops of 5 per cent. alcoholic *o*-dinitrobenzene to 5 ml. of ascorbic acid solution made alkaline by 5-8 drops of 20 per cent. NaOH, a violet colour develops rapidly, owing to reduction by the dienol grouping in the vitamin (p. 115).

Capillary Test (Göthlin, 1931-38).—Compress the upper arm for 12-15 minutes at a pressure of 10 mm. Hg above the diastolic blood pressure of the subject, by means of a sphygmomanometer cuff. If the number of petechial spots that appear in a circle 1 in. in diameter, drawn on the front of the elbow, exceeds fifteen, the subject has increased capillary fragility, which is characteristic of avitaminosis C. (Kawerau, 1942).

Vitamin P.—In 1936, Szent-Gyorgyi and his colleagues found that lemon juice was effective in curing capillary hæmorrhage of the purpura type, whereas ascorbic acid was not. The agent is a flavone glucoside and functions by increasing the resistance of capillary walls to the application of pressure. It may be obtained from lemon or orange peel, and is available in many fruits and as the commercial preparation "citrin."

P can be estimated by a capillary-resistance method (Bacharach and Coates, 1943).

MISCELLANEOUS FACTORS IN THE DIET

The Hæmopoietic Factor.—From measurements of the red cell constants: diameter, volume, iron and hæmoglobin content, it is possible to classify anæmias into two groups: (1) the hyperchromic macrocytic type, in which the cells are large and fully saturated with hæmoglobin, and (2) the hypochromic microcytic type, in which the cells are small and deficient in hæmoglobin. Hypochromic anæmia is essentially an iron-deficiency disease, and responds to iron and copper therapy. Hyperchromic anæmia, which may occur in pernicious form, is due to lack of a specific hæmatogen (the extrinsic factor) in the diet, or absence of an enzyme (the intrinsic factor) which is present in normal gastric juice, and converts the hæmatogen into a *hæmopoietic factor*, which is stored in the liver. An extrinsic factor, Bc, accompanies the vitamins of the B group in distribution, and may be obtained plentifully from rice-polishings, wheat-germ, ox heart and muscle, and yeast.

Bc has been isolated from liver, and identified as a chick-growth factor, $C_9H_{10}N_3O_3$ (Hogan *et al.*, 1940, 1943). An accompanying Bc has been identified as folic acid.

Choline, $C_5H_{15}O_2N$, acquired a significance in nutrition when Best and Huntsman in 1932, showed that it had both a curative and a preventive action on the excessive accumulation of fat in the mammalian liver. Choline is a normal constituent of the mixed human dietary, being derived from lecithin and the other phospholipides present in animal foodstuffs rich in fat. Lecithin contains about 14 per cent of choline, and its principal sources are egg-yolk, liver, kidney, heart and pancreas. Human milk has a lecithin

percentage of 0.02-0.08, while cow's milk has 0.06-0.11. Betaine, the nitrogenous base found in beetroot and other vegetable sources, has a similar effect on hepatic liposis as choline, and it has been suggested that both these compounds be listed among the water-soluble vitamins.

Young rats on a diet poor in choline develop hæmorrhagic enlargement and degeneration of kidneys, enlargement of spleen, and regression of thymus. This can be averted by doses of choline, betaine or methionine too small to influence deposition of liver fat. The severity of the condition is aggravated by cystine, fat, or cholesterol; and is ascribed to a deficiency of methyl groups available for metabolism. As du Vigneaud has shown (1941), choline, betaine and methionine are concerned in $-\text{CH}_3$ transfer, and thus protect the organism from "amethylosis."

Polyene Aliphatic Acids.—Feeding experiments by the Burrs (1929), Evans, and others, have shown that small amounts of *linoleic*, *linolenic*, or *arachidonic* acid are necessary for normal growth rate and protection from dermatitis. These acids, termed *polyene* because they contain more than one unsaturated, or *ene* linkage in the molecule, are present in all animal fats and in vegetable "drying oils." How far they can be regarded as typical vitamins is a matter of definition. Their structure is given on p. 368.

DIETARY CONSTRUCTION

Knowledge of human dietary requirements has been gained by three methods: (1) Observation and statistical analysis of the diets of civilised and primitive communities; (2) experimental study of the growth-rate and health of individuals and groups on normal and artificial diets; (3) chemical analysis of foodstuffs. The information thus accumulated is vast, and has been augmented by the world-wide, though involuntary, experiences of nations stricken by the methods of totalitarian warfare.

The construction of an individual diet involves:—

- (1) Calculation of the calorie requirements of the subject, as indicated by his age, condition and occupation.
- (2) Calculation of the minimum protein requirement. This is supplemented, as far as circumstances allow, to provide a good safety margin. The daily calorie and protein requirements represent levels which must be reached, however the dietary be planned.
- (3) Calculation of the lipide requirement, which is usually assumed to be at least equal in weight to the optimal protein requirement.
- (4) Calculation of the carbohydrate intake necessary to supplement the calories provided by the proteins and lipides, so as to bring the total up to the full calorie requirement.

(5) Calculation of the essential micro-nutrients required : namely, the vitamins, provitamins and biological elements. In practice, these are usually taken to be : vitamin A and carotene, B₁, B₂, C, D, the metals Ca and Fe, and the non-metals PO₄ and I. The residual vitamins of the B group, and the elements Na, K, Mg, Cu, Mn, Zn, along with Cl, S and, possibly, F, are sufficiently represented in a good mixed dietary not to require special consideration.

(6) Survey of the subject's normal diet to see how far it conforms to the calculated requirements ; with rectifications, where necessary, by inclusion or exclusion of particular nutrients.

The material needs of mankind are reasonably well known, and the difficulties of dietary construction are economic and social rather than biochemical. Dietary inadequacy is usually revealed by subnormal growth in the young child, by subnormal weight in the adult, and by the evidence of diseases due to lack of specific nutrients. In practice, these conditions are inter-related, as a dietary is rarely deficient in only one essential constituent.

Tables showing the relationship between weight and height are surveyed by the Nixons (*Text-book of Nutrition*, London, 1938), and are found in other standard works on nutrition.

Dietary Composition
Optimal and Marginal Daily Intake
(Bacharach and Drummond, 1940)

	Optimal.	Marginal.
Kilocalories . . .	3,500	3,000
Protein : total . . .	100 gm.	50 gm.
animal . . .	50 gm.	30 gm.
Fats	120 gm.	50 gm.
Carbohydrates . . .	485 gm.	550 gm.
Calcium	1.5 gm.	0.75 gm.
Phosphate, as P . . .	1.5 gm.	1 gm.
Iron	20 mg.	10 mg.
A and carotene . . .	7,000 i. units	3,000 i. units
B ₁	1,000 i. units	350 i. units
B ₂	2 mg.	?
C	75 mg.	25 mg.
D	400 i. units	200 i. units

This representative daily requirement for an average man in any age group is sometimes termed a "man-value" unit.

Food Composition Tables.—The value of all common foodstuffs in terms of energy and nutrients are available in tabulated surveys,

of which that of McCance and Widdowson (1940) is the most comprehensive. Abbreviated tables, modified to meet the requirements of various conditions have been compiled by Orr (1937, 1940), Mottram and Graham (1940), Cathcart and Murray (1936), Stern (1936), Abrahams and Widdowson (1937), Mottram and Radloff (1937), Wokes (1941). Representative food composition tables are printed in the Appendix (p. 543).

Dietary Standards.—Among the most important of the modern dietaries are those drawn up by Stiebling and Ward for the United States Department of Agriculture, in 1933, the British nutritional standards prepared by the Ministry of Health in 1934, and the *Recommended Dietary Allowances*, adopted by the Council of British Societies for Relief Abroad (1945).

Daily Food Requirements (Stiebling)

Subject.	Energy, kilocal.	Protein, gm.	Ca, gm.	P, gm.	Fe, mg.	Vitamin A.	Vitamin C.
						International Units.	
Child under 4 years .	1,200	45	1.0	1.0	6-9	1,500	1,085
Boy, 4-6; girl, 4-7 .	1,500	55	1.0	1.0	8-11	1,500	1,160
Boy, 7-8; girl, 8-10 .	2,100	65	1.0	1.0	11-15	1,750	1,230
Boy, 9-10; girl, 11-13 .	2,400	75	1.0	1.2	12-15	1,750	1,300
Woman, moderately active .	2,500	75	1.0	1.2	13-15	2,000	1,370
Boy, 11-12; girl, 13 .	2,500	75	1.0	1.2	13-15	2,000	1,370
Woman, very active .	3,000	75	0.88	1.32	15	2,000	1,450
Active boy, over 15 .	3,500	75	0.88	1.32	15	2,000	1,450
Man, moderately active	3,000	67	0.68	1.32	15	2,000	1,450
Man, very active	4,500	67	0.68	1.32	15	2,000	1,450

The calorie requirements of the British standards are similar to those prescribed by Stiebling; the protein requirement is specified as 80-100 gm. *per diem*, of which not less than one-third must be of animal origin.

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CHAPTER 15

ALIMENTARY DIGESTION

ALIMENTARY digestion is the process whereby the ingested food materials are rendered *assimilable*, or capable of passing through the intestinal mucosa into the portal blood stream or the lymphatic system. In general, it consists of a series of enzyme hydrolyses that convert the colloidal food molecules into simple, non-colloidal solutes or diffusible units.

Digestion cannot be studied adequately apart from physiology. It is a complex process, involving nervous and chemical co-ordination of secretion and muscular movement.

Three stages may be recognised: *oral*, *gastric*, and *intestinal*. Each is associated with the presence of specialised secretions.

ORAL DIGESTION

The digestive secretion is **Saliva**, the mixed product of three pairs of glands. It is a lubricant for the mouth cavity, a solvent, an adhesive for food material, and also has a hydrolytic action on starch, which may persist for some time after the food has reached the stomach.

Mixed saliva has a reaction of pH 5.7–pH 7.0; the usual range being pH 6.35–pH 6.85. This slight acidity may be increased by fermentation of carbohydrates in an infected mouth, and for this reason morning saliva is often the more acid. The total solids amount to about 0.6 per cent., but wide variations occur. Parotid saliva is usually more dilute than sublingual or submaxillary saliva. The total volume secreted by the normal adult in twenty-four hours can be as high as 1,500 ml., or about 1 ml. a minute.

Mixed Human Saliva (Representative Composition)

Organic Solutes, per cent.		Inorganic Solutes, Mg. per 100 ml.	
Mucin	0.2–0.3	K ⁺	30–100
Ptyalin	+	Na ⁺	20–30
Urea	0.02	Ca ⁺⁺	8
Thiocyanate	0.00–0.1	Mg ⁺⁺	1
		Cl ⁻	40–50
		H ₂ PO ₄ ⁻	10–60
		HCO ⁻	60

Identification of the Constituents of Saliva.—(i.) *Ptyalin* (salivary amylase). Add 1 ml. of saliva to 10 ml. of neutral 1 per cent. starch solution. Mix and incubate at 40°–50° C. in a water-bath. Every ten minutes withdraw a few drops of the mixture and add to one of a set of test tubes containing 5 ml. of water, 5 drops of 1 per cent. iodine reagent, and 2–3 drops of glacial acetic acid.

As zymolysis proceeds, the opalescence of the starch solution clears, and the colour reaction with the iodine changes from blue to red-brown (dextrin stage), and, finally, addition of the iodine causes no colour change (sugar stage). The original mixture will now be found capable of reducing Fehling's or Benedict's reagent, owing to production of maltose from the starch, and will give a positive test with methylamine. *Ptyalin* acts on starch and glycogen, converting them ultimately into maltose. Carbohydrate food is not kept long enough in the mouth for this change to take place, but the zymolysis continues in the stomach until the saliva is inactivated by the acid of the gastric juice. *Ptyalin* is not essential for digestion, and is absent from the saliva of many animals.

(ii.) *Mucin*.—Apply the protein copper test and the arginine test to saliva. Positive results are obtained indicating the presence of protein. To identify the protein, acidify 3–5 ml. of saliva with a few drops of 20 per cent. acetic acid as long as a precipitate forms. The precipitate is glycoprotein, and does not dissolve in excess of the acid, which distinguishes it from nucleoprotein.

Mucin is a mixture of glycoprotein and mucopolysaccharide. It acts as a lubricant and protective agent for the alimentary tract.

(iii.) *Thiocyanate*.—Add a drop or two of dilute (1 per cent.) ferric chloride to about 2 ml. of saliva. An orange-red colour developing shows the presence of thiocyanate.

Thiocyanate or sulphocyanide is secreted as SCN^- , the amount is variable and often it is absent.

Speculation has ascribed several functions to salivary thiocyanate: (a) an oral antiseptic, (b) an activator of *ptyalin*, (c) a form in which —CN compounds are eliminated, (d) a by-product of sulphur metabolism, possibly related to the taurine of bile.

(iv) *Nitrite*.—Add a couple of drops of 2 per cent. benzidine in glacial acetic acid to about 2 ml. of saliva. A yellow colour develops if the saliva contain nitrite.

This test must not be confused with the familiar benzidine-peroxide test for blood (p. 459), which yields a blue colour. Many other reagents, such as α -naphthylamine, may be substituted for the benzidine, with better results.

Saliva usually contains not more than 1–2 parts HNO_2 per million.

Ammonia and *nitrite* occur in traces in most samples of saliva from the mouth but not in catheter specimens fresh from the parotid duct. They arise from the bacterial decomposition of food residues, and their concentration is an index of oral infection.

(v) The *chloride* content of saliva is usually a little less than that of the blood plasma. It is greatly increased in conditions of chloride retention, especially those associated with acute nephritis.

(vi) *Potassium*.—Unlike the usual tissue fluids, saliva is richer in potassium than in sodium.

(vii) *Calcium* occurs chiefly in submaxillary and sublingual saliva, and aids in the formation of scale deposits on the teeth of the lower jaw. Calcium may function as an activator of ptyalin.

(viii) *Urea* is present in saliva by simple diffusion from the blood stream, as it is a very mobile solute. The urea content of saliva is about 10 per cent. lower than that of the blood, and may be estimated as a substitute for blood urea estimations, but the method is not entirely satisfactory, as there are wide variations in the composition of mixed saliva.

GASTRIC DIGESTION

Gastric juice has a double origin, the organic solutes being derived from the tubular glands lining the greater part of the stomach, while the free acid is secreted by the parietal or border cells found in the glandular epithelium.

Parietal secretion is evoked by histamine injection, and is essentially an isotonic solution of HCl, approximating in value to 0.1 N, or 0.365 per cent. Non-parietal secretion is evoked by pilocarpine injection, and is rich in nitrogenous solutes and neutral chloride, but poor in free H^+ . As collected, gastric juice may have a concentration of HCl up to 0.1 N, in man; and 0.17 N, in dogs. The pH is 1.1–1.8 in the adult, and 5 in the infant.

Significance of the Constituents of Gastric Juice.—(i.) *Pepsin* is a proteoclastic enzyme that in acid solution converts higher proteins first into acid metaproteins, and then into peptides. It is unable to attack keratin, and for this reason drugs intended to act in the intestine are sometimes administered in keratin capsules so as to pass through the stomach unchanged.

(ii) *Rennin* converts soluble caseinogen into a modification (paracasein) that forms an insoluble coagulum with Ca^{++} . It is an important enzyme in the stomach of young mammals, as by coagulating the ingested milk it retains casein in the stomach and thus prolongs the action of pepsin.

Percentage Composition of Mixed Gastric Juice

Organic Solutes, 0.04-0.15.	Inorganic Solutes, 0.08-0.5.
Pepsin + Rennin + Lipase + Hæmatinic factor + Mucin + (Pepsinogen \pm)	Total Cl . . . 0.3-0.5 H ⁺ as HCl . . . 0.2-0.36 Na ⁺ . . . 0.05-0.07 K ⁺ . . . + Ca ⁺⁺ . . . 0.003 Mg ⁺⁺ . . . + NH ₄ ⁺ . . . \pm

(iii) *Lipase*, the esterase that splits fats, does not find its optimal pH in the stomach, and does not act much on fat during gastric digestion.

(iv) *Free Acid*.—Its high acidity distinguishes gastric juice from all the other animal secretions. The concentration is usually such that one volume of pure juice will neutralise the same volume of N/10 NaOH, the result being expressed as the *titration value*. During digestion the acidity is decreased by union with digestion products, and the value of ordinary gastric contents is such that 100 ml. are neutralised by 5-50 ml. of N/10 NaOH.

Gastric H⁺ has several functions: it is necessary for the action of pepsin; it hydrolyses (inverts) sucrose, maltose, and lactose; it inactivates salivary ptyalin; it aids in the control of the pyloric sphincter; and it is an important gastric antiseptic.

When the concentration of H⁺ is low, its antiseptic action is weak, and secondary fermentations are liable to occur in the stomach. These are due to organisms swallowed along with the food, and the usual end-products are butyric acid and lactic acid (derived from carbohydrates). Butyric acid causes the characteristic sour smell of regurgitated gastric contents; lactic acid has no odour.

Identification of the Constituents of Gastric Juice

(1) *Pepsin*.—Add sufficient washed fibrin or coagulated egg-white to fill the rounded end of each of three test tubes, *a*, *b* and *c*. To *a* add 5 ml. of gastric juice and 1 ml. N/10 HCl. To *b* add 5 ml. of gastric juice and enough N/10 NaOH to make it alkaline to phenol red. To *c*, the control tube, add 5 ml. of water and 1 ml. of N/10 HCl. Label the tubes, and incubate at 40-50°. Examine after thirty to forty-five minutes. The presence of the proteoclastic enzyme pepsin is shown by the gradual dissolution of the protein

in tube *a*. The protein in tube *b* is not attacked because pepsin will only work in an acid medium.

The acid in tube *c* may cause the fibrin to swell, but does not dissolve it. The test is made more delicate by using fibrin stained with congo red or with eosin, and observing the release of the dye as the protein dissolves.

(2) **Rennin.**—Add 5 drops of neutralised juice to 5 ml. of milk. Incubate at 40–50°. The milk is converted into a solid coagulum if the juice contains rennin. Excess of juice must be avoided as the pepsin present may attack the caseinogen, and aid in the formation of a clot.

(3) **Hydrochloric Acid.**—(*a*) *Gunzberg's Test.*—Evaporate to dryness in a porcelain dish or, with precautions, on a filter paper, a mixture of 2–4 drops of gastric juice and 2–4 drops of Gunzberg's reagent (2 gm. of phloroglucinol and 1 gm. of vanillin in 100 ml. of alcohol). Avoid charring the mixture. If the juice contains free HCl, a carmine stain appears when the mixture has dried. The colour disappears on cooling, and reappears on heating.

Similar colours are given by the strong acids HNO_3 and H_2SO_4 , but these are never present in gastric juice. Lactic, butyric, and similar weak organic acids give no colour with the reagent.

(*b*) *Indicator Tests.*—About one to two hours after digestion has begun, gastric contents are strongly acid liquids, with a pH value below 2, and react accordingly with appropriate indicators. To 3 ml. of gastric juice or contents add 5 drops of 0.1 per cent. *methyl violet*, and note the colour. Repeat the test, using 0.1 per cent. *thymol blue*, and 0.1 per cent. *tropæolin 00*.

Indicator.	Colour.	pH.	Inference for Gastric Contents.
Methyl violet . . .	green.	1.0–1.5	hyperacid.
Methyl violet . . .	blue.	1.5–2.5	normal range.
Methyl violet . . .	violet.	3	hypoacid.
Thymol blue . . .	red.	1.4	hyperacid.
Thymol blue . . .	orange.	1.5–2.5	normal range.
Thymol blue . . .	yellow.	2.8	hypoacid.
Tropæolin 00 . . .	pink.	2	hyperacid or normal.
Tropæolin 00 . . .	orange.	2.0–2.5	normal range.
Tropæolin 00 . . .	yellow.	3	hypoacid.

For exact work it is necessary to match the colours with standards of known pH in a comparator.

Notes on the Indicators.—*Methyl violet*, also known as “crystal violet” and “gentian violet,” is unsuitable for titrations as it is rapidly bleached in acid solutions. The indicator is convenient for rough work, and may be obtained in the form of the ordinary copying-ink pencil, the “lead” of which is dissolved in water.

Thymol blue is a triple-change indicator ; below pH 1.4 it is *red*, between pH 2.8 and pH 8 it is *yellow*, and above pH 9.4 it is *blue*. Hence it may be used for the double titration of free acid and acid salts in gastric contents. Thymol blue has the disadvantage of being affected in acid solution by mucin and by products of protein digestion (peptones and peptides), and may fail to give an accurate indication of acids when it is applied in the later stages of gastric digestion.

Töpfer's reagent (*methyl yellow*, dimethylamino-azobenzene) is used often as an indicator of gastric acidity. Its colour change resembles that of tropæolin 00, but its transition range is between pH 2.9 (*red*) and pH 4 (*yellow*).

Tropæolin-phthalein, or "t.p." indicator is prepared by mixing equal volumes of 0.1 per cent. tropæolin 00 and phenol phthalein. Like thymol blue, it has a triple change ; *red* below pH 2, *yellow* from pH 3 to pH 8.3, and *red* above pH 9.

Estimation of the Titration Acidity of Gastric Contents

The alkali-neutralising power of gastric contents depends on : (1) the free H^+ secreted in the juice, and (2) un-ionised available H^+ , provided by various buffers and weak organic acids derived from the food. Free H^+ , often described as "free hydrochloric acid," is measured conventionally by titrating a given volume to the region pH 3, with N/10 NaOH. Un-ionised available H^+ is estimated by continuing the titration to pH 8.5-9. Gastric juice may be deficient in free H^+ (*hypochlorhydria*) or have almost none (*achlorhydria*) in conditions of pernicious, and other severe anæmias, and in gastric carcinoma, dyspepsia, and other chronic diseases. Owing to the lack of sufficient acid, the gastric contents are not effectively sterilised and fermentation of carbohydrate and other food material occurs in the stomach, with production of weak organic acids. Conversely, gastric juice may contain excessive free H^+ (*hyperchlorhydria*), as in conditions of gastric ulcer. Consequently, the recognition and estimation of these two forms of gastric acid is of great clinical value. Gastric material for analysis is obtained by giving a specially prepared "test meal" and aspirating samples of the stomach contents at various intervals, by means of a "stomach tube" that can be retained in the stomach and œsophagus during the experiment. Since neither the composition of the meal nor the circumstances under which it is taken is likely to encourage the output of gastric juice, secretion may be evoked by giving 50 ml. of 7 per cent. alcohol, as the "test meal," or by subcutaneous injection of 0.75 mg. of histamine hydrochloride, which stimulates the secretion of H^+ by the oxyntic cells.

Clinical data are usually expressed in terms of (1) "free acid" and (2) "total acid," as measured in ml. N/10 NaOH required to neutralise 100 ml. of gastric contents.

(1) "*Free acid*" is found by titrating 2 or 10 ml. of the filtered mixture to pH 2.5-3.5, using the appropriate indicator.

(2) "*Total acid*" is found by continuing the titration to pH 8.5-9, and thus includes the N/10 NaOH required for the "free acid."

(3) *Un-ionised acid*, usually termed "bound HCl and organic acid," is found by subtracting the value for the "free acid" from that for the "total acid."

Representative values, in ml. N/10 NaOH per 100 ml., for gastric contents, are: "*free acid*," 20-50, during the first 3 hours of digestion, rising to 100 or more, in hyperacidity, and fluctuating about 10 in achlorhydria and hypoacidity; "*total acidity*" reaches a maximum of 60-80 about the second hour after digestion has begun, the values depending very much on the type of food taken. In conditions of hypochlorhydria, the total acidity is due, chiefly, to the un-ionised acids, and may be greatly increased by lactic or butyric products of fermentation. When a sample of gastric contents has a low "free acid" value, and a high "total acid" value, it should be tested for the presence of lactic acid.

By using an appropriate triple-change indicator both the "free acid" and the "total acid" can be estimated consecutively in the same sample.

Total Titration Acidity

	Free H ⁺		acid salts + organic acids			
	red	orange	yellow	green	blue	
Thymol blue	red	orange	yellow	orange	red	
T.p. indicator	red	orange	yellow	orange	red	
pH	1.4	2	2.8	3	8	9

(1) Measure carefully by pipette 10 ml. of gastric contents into a flask or large test tube. Add 5 drops of 0.1 per cent. thymol blue or t.p. indicator. A red colour denotes the presence of "free hydrochloric acid."

(2) Titrate the mixture with N/10 NaOH. The first end-point is reached when the red changes to orange. This can best be decided by comparing with a similar flask or tube containing 10 ml. of pH 3.0 buffer solution and 5 drops of the indicator.

Note.—A single estimation merely indicates the presence or absence of an adequate amount of free hydrochloric acid. In clinical practice, the estimation is made serially on samples (2-5 ml.) of contents aspirated

from the stomach at fifteen-minute intervals after a meal. Such data show the rate and course of gastric secretion.

Detection of Abnormal Acids in Gastric Contents

Butyric acid, $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$, can be recognised immediately by its characteristic sour smell.

Lactic Acid, $\text{CH}_3\text{CH}(\text{OH})\text{COOH}$.—(a) *In the absence of "free hydrochloric acid."*—Add just sufficient dilute (1 per cent.) ferric chloride to 10 ml. of water to make it faintly yellow. Divide the mixture into two parts. To one tube add 2–3 ml. of the gastric contents (filtered, if necessary), and compare the colour of the two tubes. If lactic acid is present, the colour deepens to greenish-yellow. Unless this test is checked against the control tube it is unreliable.

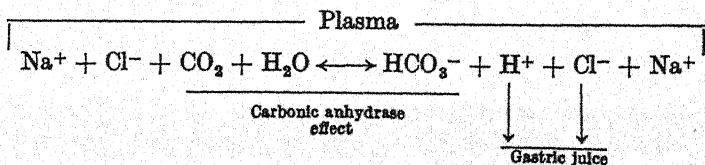
(b) *In the presence of "free hydrochloric acid."*—The mixture must be extracted with ether, and the ferric test applied to the extract.

Extract, without shaking, 5 ml. of filtered gastric contents with 20 ml. of ether, preferably in a separating funnel. Let the mixture separate into two layers. Remove 5 ml. of the ether carefully so as to avoid contamination with gastric contents. Add 10 ml. water, and about 10 drops 1 per cent. ferric chloride solution. Shake gently. If the gastric filtrate contained 0.05 per cent. lactic acid the mixture will turn greenish; over 0.1 per cent. produces a distinct yellow.

A fallacy may be introduced into either form of the test by the presence of thiocyanate from swallowed saliva. This gives a red colour with ferric chloride, but, unlike the lactate, it is bleached on addition of a few drops of 5 per cent. mercuric chloride.

Origin of Gastric Acid.—The mechanism whereby the gastric glands secrete an acid of the strength and concentration of N/10 HCl is obscure. Gastric mucosa is rich in the enzyme *carbonic anhydrase*, which may provide the necessary concentration of H^+ by hydrating to H_2CO_3 the CO_2 produced by local metabolism.

This hypothesis is supported by the fact that thiocyanate, which inhibits carbonic anhydrase, when administered, is excreted by the gastric mucosa, and greatly depresses the secretion of HCl. However, sulphanilamide drugs, which powerfully inhibit carbonic anhydrase, and also are excreted by the gastric mucosa, do not affect the output of HCl (Feldberg *et al.*, 1940).



DUODENAL DIGESTION

This is brought about by the combined action of three secretions : pancreatic juice, bile, and intestinal juice, or *succus entericus*. Digestive activity is maximal about the second stage of the duodenum, and the process continues throughout the small intestine.

Percentage Composition of Human Pancreatic Juice

Organic Solutes, 0·6-0·7.				Inorganic Solutes, 0·7.			
Trypsinogen	.	.	+	Na ⁺	0·25	Cl ⁻	+
Pancreatic erepsin	.	.	+	K ⁺	0·0008	HCO ₃ ⁻	0·5
Amylase	.	.	+	Ca ⁺⁺	0·003	HSO ₄ ⁻	+
Lipase.	.	.	+	Mg ⁺⁺	+	H ₂ PO ₄ ⁻	+
Lipides	.	.	0·52				

The pH of human pancreatic juice is about 8, and the alkalinity corresponds approximately to a 0·53 per cent., or N/10 solution of sodium carbonate, hence one volume of average gastric juice is neutralised by about the same volume of pancreatic juice.

Identification of the Constituents of Pancreatic Juice.—The most characteristic constituent of pancreatic juice is the enzyme trypsin, which is secreted in the inactive form as trypsinogen, and is activated by the enterokinase of the intestinal juice. Trypsin is identified by its proteoclastic action on fibrin in alkaline solution. Three test tubes are prepared and incubated, as in the method for identifying pepsin in gastric juice (p. 303).

To *a* add 5 ml. pancreatic juice made alkaline with N/10 Na₂CO₃.

To *b* add 5 ml. pancreatic juice, acidified with N/10 HCl.

To *c* (the control tube) add 5 ml. N/10 Na₂CO₃.

Incubate for 30-40 minutes. The presence of trypsin, the protein-splitting enzyme that operates in alkaline solution, is shown by the digestion of the protein in tube *a*.

Lipase.—This enzyme can only be found in fresh extracts of the pancreas or juice recently secreted. It is detected by the fat-hydrolysis test previously described for the lipides.

Amylase.—This enzyme is detected by the starch-hydrolysis test as described for ptyalin.

BILE

Bile may be obtained in two forms : fistula bile, the fresh secretion of the liver ; and gall-bladder bile, which has been stored and

concentrated prior to being poured into the duodenum. The daily secretion of fistula bile is fairly constant, and usually between 600 and 700 ml. in twenty-four hours. It is alkaline, whereas gall-bladder bile is neutral or slightly acid.

Percentage Composition of Human Bile

Fistula Bile.		Bladder Bile.	
Water	98	Water	89
Total solids	1.92	Total solids	11.0
Bile salts	0.715	Bile salts	6.0
Pigments	0.15	Pigments	2.0
Cholesterol	0.057	Cholesterol	0.375
Nucleoprotein	+	Nucleoprotein	+
Mucin	+	Mucin	+
Inorganic solutes	0.7	Inorganic solutes	0.8
pH	7.5-8	pH	6.5-7

Significance of the Constituents of Bile.—(i.) **Bile Acids.**—These are chiefly glycocholic acid, $C_{26}H_{43}NO_6$, and taurocholic acid, $C_{26}H_{45}NSO_7$, which are peptides of cholic acid.

In the resting state before meals, about 95 per cent. of the total bile salts of the body are collected in the gall bladder.

The bile salts are the active digestive agents in the bile secretion. They have a very bitter taste. They lower surface tension, and thus promote : (i.) emulsification of food colloids, and (ii.) intestinal absorption of lipides. During absorption, the bile salts act as hydrotropic carriers of the fatty acids, and are reabsorbed into the circulation, and resecreted by the liver, the process resulting in a continuous circulation of bile salts. When administered by the mouth, they act as a powerful natural cholagogue.

In obstructive jaundice, lack of the bile salts in the intestine gives rise to digestive disturbances due to the inability to absorb the hydrolysed fat.

Cholic acid is synthesised and esterified in the liver. Its precursors are obscure, and probably form part of the system whereby cholesterol is manufactured in the organism.

(ii.) **Cholesterol.**—With the possible exception of milk, bile is the only human secretion containing any considerable amount of cholesterol, and it is the chief channel of excretion of the sterol. The range of concentration is very great ; values of from 0.01 to 1.3 per cent. have been obtained for bladder bile, the average being 0.3,

corresponding to a daily output of 0.34–0.4 gm. Biliary cholesterol is highly important in connection with the formation of sterol calculi, a common variety of “gall stones.”

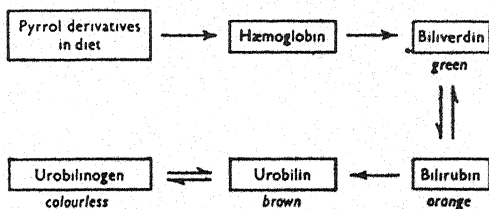
“The liver possesses the power of synthesising both cholesterol and cholic acid, and thus controls the amount of these substances present in the bile. Under normal conditions the amount of cholic acid produced synthetically is probably small, because the bile salts are almost completely reabsorbed from the intestine and return to the liver.

“The production and reabsorption of cholesterol is a much more variable process. The liver probably obtains some of its supply from the diet, also from the breakdown of old blood corpuscles. Reabsorption hardly occurs at all in some animals, while with others it is practically complete. In the intestine, cholesterol frequently undergoes more or less complete reduction to coprosterol, and this appears to be one of the factors limiting reabsorption” (Fox, 1927).

(iii.) **Bile Pigments.**—The orange-red pigment, *bilirubin*, and the green pigment, *biliverdin*, occur in varying proportions in the bile of different animals, and may be accompanied by related pigments. Human bile is golden yellow owing to the presence of bilirubin. Ox bile is green owing to biliverdin and phylloerythrin. Phylloerythrin, as Marchlewski has shown, comes from the chlorophyll of the diet (p. 218). Bilirubin and biliverdin are waste products derived from hæmoglobin, and are formed in the reticulo-endothelial tissue lining the vascular channels of the liver and the spleen, and elsewhere. Extrahepatic pigment is transported to the liver by the blood stream, which has a bilirubin content of 0.1–0.5 mg. per 100 ml. In conditions of obstructive jaundice, bilirubin accumulates in the blood until the renal threshold limit of 2 mg. per 100 ml. is exceeded, when the pigment overflows into the urine.

Conversion of hæmoglobin into bile pigment involves three stages: (1) formation of a *pseudo*-hæmoglobin in which the porphyrin ring is opened yielding verdohæmoglobin, (2) removal of globin and Fe^{++} yielding biliverdin (Lemberg, 1942); (3) reduction of biliverdin to bilirubin. Free porphyrins, which are toxic, are not released during the conversion. The pigments enter the intestine in the bile, and are not reabsorbed, but undergo bacterial reduction to urobilins which make up the stercobilin pigment that colours the intestinal contents. When there is excessive formation of urobilin, some of it is absorbed, and, in part, reduced to urobilinogen, which is excreted by the kidney. On oxidation, urobilinogen forms urobilin, the brown pigment of the urine.

Inter-relationship of the Bile Pigments



Fæcal and urinary urobilin, $C_{33}H_{46}O_6N_4$, is lævorotatory, unlike the inactive urobilin got by atmospheric oxidation of *mesobilinogen*, the primary chromogen formed when bilirubin is reduced by Na amalgam or by bacteria. Watson (1942) concludes that a fæcal factor is concerned in producing normal urobilin, or stercobilin. Mesobilinogen may occur in pathological urines.

Identification of the Chief Constituents of Bile.—I. Pigments

Note the characteristic orange colour due to bilirubin, and the viscosity due to nucleoprotein and mucin. Fresh bile is slightly alkaline, but its colour is liable to obscure the indicator. For the purposes of the following tests pig's bile is used, previously diluted with water, about 1 : 20 :—

Extract 5–10 ml. of dilute bile with about 3 ml. of ether or toluene. No pigment is removed if the bile be fresh. Acidify the mixture with a couple of drops of concentrated hydrochloric acid, and mix by inversion. The ether or toluene is coloured yellow by the liberated bilirubin.

(1) **Oxidation Tests.**—Gmelin's test. Add slowly, by pipette, 2–4 ml. of dilute bile so as to form a layer on 2 ml. of yellow, "fuming" nitric acid in a test tube. At the liquid junction, zones of coloured oxidation products develop, ranging from mesobiliverdin (green), mesobilicyanin (blue), to red choletelin. Alternatively, 2–3 drops of dilute bile are added to 5 ml. of Hammarsten's reagent, which is made by mixing 1 ml. of 25 per cent. HNO_3 with 19 ml. of 25 per cent. HCl , and after 24 hours diluting with 80 ml. of 95 per cent. alcohol. On shaking up with the bile, the characteristic green colour develops, and later changes to blue.

Other oxidation tests, employing iodine, or turpentine, are described for the detection of bile pigment in urine (p. 460).

(2) **Diazo Test** (van den Bergh).—Add 2 ml. of alcohol to 2 ml. of dilute bile. Mix and add 1 ml. of fresh diazo reagent (p. 548). A red colour develops owing to the presence of bilirubin. Acidify with hydrochloric acid. The colour changes to violet.

The reagent in acid solution is a specific test for bilirubin, and gives no colour with biliverdin. The reaction is the basis of the standard method for estimating bile pigment in blood plasma by comparing the colour with a control containing a known amount of bilirubin. Values are measured in units, each of which represents 1 part of bilirubin in 200,000, or 0.5 mg. per 100 ml. Fistula bile has a value of about 50 units, or 25 mg. per 100 ml.; blood plasma has about 0.2–0.5 units in health, but may exceed ten times this amount in obstructive jaundice, and in toxic conditions leading to increased destruction of erythrocytes.

In the blood, bilirubin is united to serum albumin, and only gives an "indirect reaction" with the diazo reagent. On reaching the liver it is converted into free bilirubin prior to excretion in the bile.

II. Bile Acids (Taurocholic and Glycocholic Acid)

(1) *Sulphur Test* (Hay).—This test, which demonstrates the lowering of surface tension by bile acids, is described later as a test for bile in urine.

(2) *Sugar Test*.—Mix a drop of undiluted bile with 5 ml. of concentrated HCl. The oxidised pigment colours the solution red. Boil gently until most of the colour is discharged. Remove the flame, and add a very small fragment of sucrose. Mix well. The hot solution gradually turns bright purple.

This test resembles the thymol test for carbohydrates. The phenol is replaced by cholic acid, which combines with the furfural set free from the sugar (p. 126). A slight excess of sucrose forms a brown colour that obscures the test.

III. Proteins

When bile is acidified a dense precipitate appears, consisting of *nucleoprotein*, which is soluble in glacial acetic acid, and *mucin*, which is insoluble in the acid. The proportions of these proteins differ in different animals. Ox bile is rich in nucleoprotein, but poor in mucin. By careful precipitation the proteins can be separated, filtered off, washed, and redissolved in sodium carbonate solution. They give the tests characteristic of their type (p. 142).

IV. Cholesterol

There is no simple direct test for cholesterol in bile. It is kept in solution in bile by the bile acids, but can be identified in biliary calculi, or in the residue from the alcoholic extraction of bile dried on a water-bath, by the characteristic shape of the crystals and by the sterol colour tests (p. 191).

INTESTINAL JUICE

The mixed secretion of the small glands of the duodenum, jejunum and ileum constitutes the intestinal juice, or succus entericus. As obtained from experimental fistulæ, it is an alkaline liquid of pH 7.1 (duodenum) to 7.6 (ileum), and contains a great variety of enzymes, which complete the hydrolyses begun by the catalysts of the earlier secretions.

Composition of Dog's Intestinal Juice

Organic Solutes, 0.6-0.7 per cent.				Inorganic Solutes, 0.7 per cent.	
Erepsin complex	+	Maltase	+	NaCl	0.5
Nucleinase	+	Lactase	+	Ca ⁺⁺	+
Nucleotidase	+	Sucrase	+	Mg ⁺⁺	+
Enterokinase	+	Amylase	+	HCO ₃ ⁻	+
Esterases	+				

The intestinal mucosa contains important *phosphatases*, which phosphorylate the hexoses prior to their absorption, and a *nucleosidase*, which hydrolyses the nucleosides derived from the nucleoproteins (p. 142).

Summary of the Digestive Process

Region.	Secretion.	Reaction.	Enzymes.	Substrates.	End-products.
Mouth	Saliva.	Slightly acid or neutral; pH 5.8-7.0.	Ptyalin (amylase). Maltase.	Starch. Dextrin. Glycogen. Maltose.	Maltose. Glucose.
Stomach	Gastric juice.	Acid; pH 1.7.	Pepsin. Rennin. Lipase.	Proteins. Caseinogen. Fats.	Peptides. Casein. Aliphatic acid + glycerol.
Intestine	Pancreatic juice.	Alkaline; pH 8-10.	Trypsin. Erepsin. Amylase. Maltase. Lipase.	Proteins. Peptides. Starch, etc. Maltose. Fats.	Peptides. Amino acids. Maltose. Glucose. Acid + glycerol.
	Bile.	Alkaline; pH 8.	Phosphatase	Organic phosphates	H ₂ PO ₄ .
	Intestinal juice.	Alkaline; pH 7-8.	Erepsin. Sucrase. Lactase. Nucleinase. Nucleotidase. Lipase. Enterokinase.	Peptides. Sucrose. Lactose. Nucleic acids. Nucleotides. Fats. Trypsinogen.	Amino acids. Glucose + fructose. Glucose + galactose. Nucleotide. Nucleoside. Trypsin.
Intestinal mucosa.	—	—	Nucleosidase. Phosphatase.	Nucleosides. Hexoses.	Purines + sugar. Hexose phosphate.

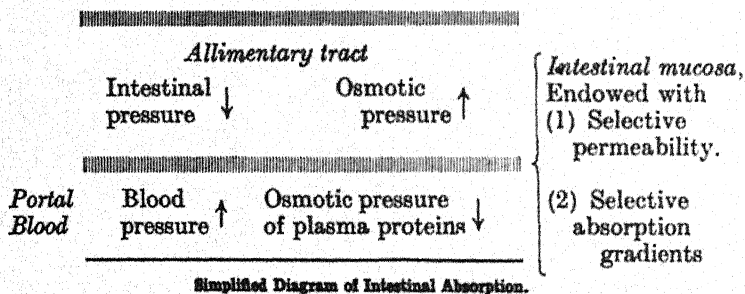
INTESTINAL ABSORPTION

The efficiency of the human digestive process in health is such that about 96 per cent. of the total mixed dietary is made available for absorption into the portal and lymphatic systems. Colloids have been converted into diffusible solutes, insoluble fats have been emulsified and saponified, compound saccharides have been changed into utilisable monosaccharides, and foreign proteins have been rendered non-toxic by being hydrolysed into non-specific mixtures of amino acids.

The faculty of absorption resides in the entire alimentary tract, being maximal in the small intestine. Absorption in the mouth and oesophagus is negligible, gastric absorption is almost entirely restricted to alcohol and CO_2 , colonic absorption is chiefly water.

For a nutrient to be absorbed, either it must be water-soluble and of low molecular weight, or must be combined with a carrier such as the bile acids. The first form of absorption is almost indiscriminate; the second form shows high discrimination with regard to certain reactants.

Absorption of Aqueous Solutes.—The chief factors determining the absorption of water and simple solutes are diffusion and osmosis. The absorption of a solute follows its diffusion gradient, or relative concentrations in the two media. When the concentration is greater in the intestine than in the blood it moves from the intestine. Water is absorbed as soon as the solution in the intestine has a lower osmotic pressure than that of the blood.



Martin Fischer, however, has emphasised the importance of the water-binding property of the tissue colloids, which enables them to act as carriers for solutes, thus setting up absorption gradients.

An isotonic solution of glucose or sodium chloride loses its solute so rapidly that it becomes hypotonic, and absorption of the solvent occurs. An isotonic solution of a non-diffusible solute, such as

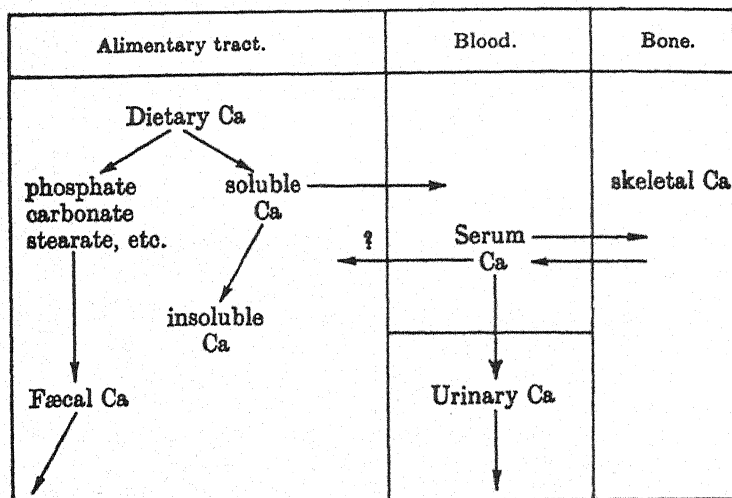
magnesium sulphate, or a sugar of low diffusibility, such as xylose, remains unchanged for a long time in the intestine ; while a hypertonic solution of these compounds can act as a cathartic by abstracting water from the blood, and thus diluting the intestinal contents. The biological hexoses in particular, are rapidly absorbed owing to the presence of phosphatase in the intestinal mucosa, which transforms them into phosphoric esters, and thereby increases the diffusion gradient.

Proteins are absorbed in the form of amino acids and also, to an uncertain extent, as simpler polypeptides (London and Kotschneff, 1928-34). Absorption of intact protein by a defective mucosa is responsible for allergy, or hypersensitiveness to certain foodstuffs, such as egg-white and milk protein.

Absorption of Inorganic Ions : Calcium.—The mechanism of calcium absorption is obscure, and it is doubtful if conclusions drawn from studies on lower animals can be applied directly to the human subject. In the average pH range of 6-7, the contents of the human intestine are insufficiently acid to keep calcium from being precipitated as phosphate or soap, although some free Ca^{++} may exist in the acid zones of the jejunum, and be absorbed as such. Calcium absorption may depend on the production of a diffusible non-ionised complex, such as is formed with citric acid, which explains why citrus fruits, although free from vitamin D, promote calcium uptake. A soluble complex is also formed by bile acids and calcium soaps. Vitamins of the D group promote the absorption of calcium, but the action is indirectly effected through phosphate-balance control. After absorption, about 0.5 to 1 gm. of Ca^{++} is returned to the intestine daily in the digestive secretions, but this is reabsorbed again. Calcium absorption is retarded by calcium precipitants present in the ordinary mixed diet, and promoted by proteins or amino acids.

Precipitants include *fatty acids*, when the fat eaten is in excess ; *phosphates* ; *phytic acid*, from bran and entire cereals ; *oxalic acid*, from fruits (strawberry, gooseberry) and vegetables (rhubarb, sorrel), or from fermentation of carbohydrate in the stomach. As a result, even under favourable conditions, about three-quarters of the total calcium of the dietary may escape absorption ; while, if the calcium precipitants are in excess or if the diet be poor in calcium or vitamin D, not only will no calcium be absorbed, but the calcium in the digestive secretions may also be lost, resulting in a state of negative calcium unbalance, and decalcification of the body.

FACTORS REGULATING CALCIUM ABSORPTION



Intestinal Ca Output increased by :

- (1) Excess Ca in diet.
- (2) Excess of P in diet.
- (3) Alkaline dietaries.
- (4) Excess of fat in diet.
- (5) Phytic acid in diet.
- (6) Lack of vitamin D.

Renal Ca Output increased by :

- (1) Excess Ca in diet.
- (2) Deficit of P in diet.
- (3) Acid dietaries.
- (4) Starvation.
- (5) Parathyrin injection.
- (6) Any other condition tending to raise the serum Ca level.

Phosphate.—Tracer studies, using PO_4^{3-} labelled by the radioactive isotope, ^{32}P , show that about two-thirds of the daily phosphate intake of 1–1.5 gm. P are absorbed, the rest being excreted as calcium and magnesium phosphate. Once absorbed or injected, very little phosphate is re-excreted by the intestine. Phosphate present in the diet as phytic acid or phytates, the chief phosphorus compounds of cereals, is almost unavailable for human nutrition, what is released comes from the action of phosphatases present in the intestinal micro-organisms, or in the yeast used in bread-making, or already present in the cereal.

Iron.—Gastric HCl releases iron from the foodstuffs and it is reduced to the ferrous form, Fe^{++} , in which it is absorbed in the duodenum. Reduction is probably by cysteine or thiol groups liberated during digestion of proteins, though ascorbic acid also may act. Iron absorption is determined by the iron needs of the organism, and is increased in conditions of anaemia. Absorption is

retarded by precipitants, such as phosphate and sulphide. Food iron in the form of hæmoglobin and the hæm pigments of muscle is poorly absorbed, and a practical distinction is drawn between sources of *available* iron, such as liver, kidney, entire cereals, and green vegetables, and sources, such as lean meat, that in spite of their reputation and appearance, contain iron chiefly in a *non-available* form. Once absorbed, iron is not excreted to any significant extent either by the intestine or the kidneys, although, for some unknown reason, this conservation does not lessen the need for a dietary supply of the metal.

Absorption of Lipides.—In the small intestine, where absorption is most active, the pH of the contents is usually acid, and the fatty acids are present as such, since soaps form when the pH is >8 . These fatty acids are emulsified and made water-soluble by the hydrotropic action of the bile acids and bile lecithin, and in this form are absorbed into the lymphatics of the intestinal villi, and pass into the thoracic duct as *chyle*, a milky emulsion containing 4 to 6 per cent. of neutral fat, which shows either that fat is absorbed unchanged, or that reconstruction of fat from acid and glycerol must have taken place in the villi during absorption.

The fat particles, or *chylomicrons*, have a diameter of 0.05 to 1μ . They can be counted microscopically in a drop of chyle or blood plasma, two hours after a meal rich in fats. About 80 per cent. of fat absorbed can be recovered by collecting the chyle from the lymphatic ducts before it reaches the general circulation. According to the fat-partition theory of Frazer (1940), unhydrolysed fat when finely emulsified can pass directly into the lymphatic system, and thus enter the general circulation, whereas fatty acids from hydrolysed fat are absorbed by the capillaries, enter the portal vein, and thus reach the liver. Hence, the degree of lipolysis in the intestine determines the distribution of fats between the adipose depots in the tissues and the metabolically active liver. Three factors are concerned in fat absorption: (i.) the enzyme lipase and its activators; (ii.) the bile constituents which act as carriers; (iii.) the phosphorylation enzymes in the mucosa. This elaborate mechanism endows the organism with considerable power of discrimination in the absorption of lipides and lipoids, as shown by the preferential absorption of carotene.

Man absorbs cholesterol more readily than the related sterols, but shows no preference for any one of the carotinoids. The cow absorbs carotene and excludes xanthophyll, although the latter predominates in herbage. The absorption of carotinoids and vitamin A is greatly facilitated by the presence of fats in the dietary.

Intestinal Excretion.—The waste material eliminated by the human intestine varies greatly in composition and amount; the daily output of 100 gm., being raised to 200 gm., or more, by consumption of foodstuffs rich in cellulose and fibre, such as leafy vegetables and high-extraction cereals, or brown bread. Taking 100 gm. as an average output, this represents about 20–40 gm. of total solids, which contain 2–3 gm. of nitrogen and are derived from: (1) epithelium, mucin and other secretions of the alimentary tract; (2) unabsorbed food residues, chiefly cellulose, and soaps of Mg and Ca; (3) steroids, chiefly from bile; (4) bacteria and other micro-organisms, most of which are dead. Faecal lipoids, representing some 10 per cent. of the total solids, are defined as the ether-soluble fraction, and include coprosterol and other steroids derived from bile cholesterol, as well as unabsorbed steroids from the diet. Faecal pigment is chiefly stercobilin, formed by bacterial reduction of bilirubin. Volatile constituents include indole, scatole, H_2S and thiols, and traces of lower fatty acids, such as valeric.

Micro-organisms make up 10 to 20 per cent. of the faecal solids, and are represented by: (1) acid-forming types, such as *Lactobacillus acidophilus*, *L. bifidus*, *Bact. coli* and *Bact. aerogenes*, all of which ferment sugars, forming lactic and other acids, and gases, such as CO_2 , H_2 and CH_4 ; (2) proteoclastic or putrefactive types, chiefly *Streptococcus faecalis* and *Clostridium welchii*, which decarboxylate and dehydrogenate amino compounds. The metabolic activities of these organisms are determined by the composition and pH of the intestinal contents, which, in turn, depend on the dietary. Thus, a preponderance of carbohydrate, especially the slowly assimilated sugar lactose, favours the growth of acid-forming types and, by increasing the acidity of the intestinal contents, retards the growth of putrefactive types and members of the colon group, including *Bact. coli*, the dominant organism of the intestine.

Biological Significance of the Intestinal Flora.—While it is popularly believed that the micro-organisms that colonise the lower alimentary tract of man and other animals are only to be tolerated as undesirable but permanent aliens, evidence from nutritional studies shows that some of these parasites may contribute to the welfare of their hosts by synthesising useful vitamins. Conversely, when the alimentary tract is sterilised by administration of drugs such as sulphaguanidine, which is not absorbed from the intestine, signs of multiple vitamin deficiency have been found to develop.

Vitamins provided by the activity of the intestinal flora are believed to include: inositol, pantothenic acid, biotin, folic acid, *p*-aminobenzoic acid and, under certain conditions, thiamine.

Micro-organisms also supply the enzyme *cellulase*, or *cytase*, necessary for the digestion of the cellulose that makes up the bulk carbohydrate in the diet of cows, horses and other graminivora.

Starvation Phenomena.—The employment of systematic starvation as a political weapon has abundantly demonstrated that prolonged deprivation of food leads to degeneration of the epithelium of the alimentary tract, which both decreases the absorption efficiency of the victim and renders him more liable to ulcerations and other intestinal infections.

In treatment of such states, ordinary foodstuffs may act as irritants, and nutrients are supplied in the simplest forms as glucose and amino acid mixtures got by the trypsin or papain hydrolysis of muscle and milk proteins. Properly prepared, these protein hydrolysates should be of high nutritional value, though the presence of certain peptides may give them an unpleasant taste.

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CHAPTER 16

INTERMEDIATE METABOLISM: CARBOHYDRATES

INTERMEDIATE metabolism comprises the history of the biochemical compounds within the organism, starting with the changes undergone by the foodstuffs after absorption into the blood stream. In theory, each molecular species has its own independent career from the time it passes into the portal blood or lymphatic system, but observation shows that a physiological co-ordination, or *homeostasis*, exists controlling the chemical mechanisms of intermediate metabolism, which may be defined as *the sequence of transformations by which energy becomes available for life*.

While the food materials remain in the alimentary tract they are physiologically outside the organism; it is only after absorption they come into direct contact with the cytoplasm and participate in the operations of life.

CARBOHYDRATE METABOLISM

The carbohydrates enter the animal in the form of *monosaccharides*; as the three *hexoses*, $C_6H_{12}O_6$, glucose, fructose, and galactose, derived from the starches and sugars of the dietary, and, to a small extent, as *pentoses*, $C_5H_{10}O_5$, from nucleoproteins and pentosides.

Pentose metabolism is obscure. The organism has a low tolerance for these sugars, and they readily appear in the urine after ingestion of fruits and vegetable gums (alimentary pentosuria). At the same time they may be synthesised to meet the demands of the growing tissues for nucleoproteins; and in the condition of congenital pentosuria they are excreted continuously, irrespective of the pentoses derived from the diet.

Hexose Absorption.—The relative speed of sugar absorption from the intestine is in the order: galactose > glucose > fructose; the concentration of sugar affects the duration but not the rate of absorption, showing that it is not a simple diffusion process.

The hexoses on entering the cells of the intestinal wall are converted into the corresponding phosphoric esters, and by this means the diffusion gradient is kept favourable for sugar absorption. Pentoses, disaccharides and hexoses other than D-glucose, D-fructose, D-galactose and D-mannose are not phosphorylated by the

intestinal enzyme system, and their rate of absorption is relatively slow, and is regulated by simple diffusion.

By means of iodoacetic acid or the glycoside *phloridzin*, it is possible to inhibit the phosphorylating mechanism in the intestinal wall, and when this is done, the preferential absorption of hexoses ceases, and they diffuse in a manner similar to the other saccharides.

Hepatic Function.—All the glucose absorbed in excess of the immediate requirements of the tissues travels in company with the fructose and galactose of the diet to the liver, where they are converted into glycogen, the storage polysaccharide of the body. This process of polymerisation involves the interconversion of the three hexoses into a common unit, and when glycogen is broken down again the product is glucose. Thus the liver both transforms and stores carbohydrate.

The glycogen content of the human liver is variable, and the average value for the adult is about 3 per cent. of the fresh tissue. A meal rich in carbohydrates may raise the hepatic glycogen of a man or a rabbit up to 7 per cent., but this level is not maintained during daily activity.

The total amount of glycogen in the adult human body is assessed at an average range of 400–500 gm., approximately half being in the muscles and half in the liver. Muscle glycogen is part of a contraction mechanism, and yields lactic acid when broken down; hepatic glycogen is the storage polysaccharide of the body, and yields glucose. Hepatic glycogenolysis involves a phosphorylation mechanism, and is not due merely to liver amylase, the amount of which is too low to account for the speed of glycogenolysis.

Glycogen	←————→	Glucose
Hepatic glycogen synthesis (glycogenesis) is promoted by :—		Hepatic glycogen resolution (glycogenolysis) is promoted by :—
(1) High blood sugar level.		(1) Low blood sugar level.
(2) Insulin.		(2) Adrenalin.
(3) Corticosterone.		(3) Thyroxin.

The Blood Sugar.—The venous or capillary concentration of sugar in the post-absorptive stage (twelve to sixteen hours after a meal) is usually within the limits 70–120 mg. per 100 ml. Prolonged starvation may reduce the level to half its normal value. Immediately after ingestion of glucose (50–100 gm.) the blood sugar level rises above the fasting value in both venous and capillary blood, being lower in the former owing to the continual transfer of sugar from the blood to the tissues. The venous maximum is reached in twenty to forty minutes, about the region 130–150 mg. per 100 ml.

The arterial maximum is 30–70 mg. higher, and is reached about the same time. The value returns to normal after two hours.

The rise in blood sugar concentration is not proportional to the amount of sugar administered, and depends on a complex utilisation mechanism whereby glucose is removed from the circulating blood.

The Glucose Threshold.—When the glucose concentration in the blood rises to a critical maximum, between 174 and 216 mg. per 100 ml. in health, sugar is excreted in the urine, the condition being one of hyperglycæmic glycosuria. The maximum is termed the "rising glucose threshold," the implication being that as soon as the sugar concentration exceeds a certain limiting value it overflows into the urine.

Glucose is an essential metabolite, and has a high renal threshold level. It passes through the glomeruli, along with the other filtrates from the plasma, and is re-absorbed by the convoluted tubules. Renal absorption of glucose is so efficient that only traces of the sugar appear in normal urine. The absorption is determined by the formation of phosphoric esters in the tubule cells, and is comparable to the absorption of glucose from the intestine. Foreign sugars, such as pentoses, which do not undergo esterification by phosphatase, pass along the tubule into the urine, where they contribute to the reducing substances normally present. If the blood glucose exceeds a limiting value, or if the renal absorption mechanism is defective, the filtrate is not freed from glucose, and a condition of true *glycosuria* results. By injection of phloridzin in doses of above 0.5 gm., it is possible completely to inhibit the renal absorption. In this condition of *phloridzin diabetes*, the renal threshold for glucose is lowered almost to zero, and glycosuria results, until the animal reaches a state of severe hypoglycæmia, owing to depletion of its carbohydrate reserves.

The average *true* sugar value for human blood obtained after a night's rest is believed to be 80 (\pm 20) mg. per 100 ml., and is due to a mixture of α - and β -glucopyranose. The *total reducing* value of blood, as found by the usual analytical methods, is much higher, and when expressed as glucose, or "blood-sugar," is about 120 mg. per 100 ml., while 180 mg. per 100 ml. is approximately the renal threshold level in the human subject.

Blood Sugar Curves.—Increase in the blood-sugar content, as the result of ingestion of carbohydrate or injection of glucose, evokes a compensatory secretion of insulin, which lowers the blood-sugar level, by: (1) promoting glucose uptake by the peripheral tissues, (2) promoting glycogen formation in muscle, and (3) retarding glycogenolysis in the liver.

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By analysis of small samples of fresh liver removed from thirty-three patients during abdominal operations, MacIntyre (1941) found the glycogen percentage to range from 1.1 to 6.3, the average being 3.15. Administration of glucose beforehand raised the average levels to 4.7-5.0.

The extent and duration of the hyperglycæmia following glucose administration is a valuable clinical index of the insulin output and efficiency of the pancreas, provided that the subject has been on a uniform diet.

Representative Blood Sugar Values (Expressed in mg. glucose per 100 ml.)

Subject.	Time in minutes after ingestion of 50 gm. glucose.				
	0	30	60	90	120
Normal Adult :					
Diet A .	100	120	130	110	100
Diet B .	100	118	118	110	120
Diet C .	100	170	200	160	145
" Lag " type .	100	150	230	140	120
Diabetic :					
Mild .	170	187	198	190	182
Severe .	240	270	294	300	314
Renal glycosuric	98	100	94	96	98

Diet A included 300 gm. carbohydrate daily during the previous week ; diet B included 500 gm. carbohydrate and 40 gm. fat ; diet C included 50 gm. carbohydrate and 240 gm. fat. These observations by Himsworth (1935) show that a high-fat low-carbohydrate diet can repress the pancreatic response in the normal subject, and produce a sugar-tolerance curve resembling in rise and prolongation that of the diabetic. Hence, for accurate assessment of clinical states the subject should have been on a diet containing at least 300 gm. of carbohydrate for not less than three days before the sugar-tolerance test. The " lag " type of curve is usually accompanied by alimentary glycosuria, and denotes that the subject's capacity for sugar storage is sub-normal in relation to his sugar-absorption rate.

THE GLYCOTROPIC HORMONES

The blood sugar level is maintained in accordance with the needs of the organism by the co-action of five hormones : adrenaline, corticosterone, insulin, and the glycotropic factors of the anterior pituitary gland.

Adrenaline (adrenine), or epinephrine, the hormone of the adrenal medulla, influences carbohydrate traffic in three ways :—

- (a) Acceleration of hydrolysis of liver glycogen to glucose, an effect antagonised by insulin.
- (b) Acceleration of conversion of muscle glycogen to lactate.
- (c) Retardation of glucose acceptance by peripheral tissues.

Corticosterone.—Removal of the adrenal cortex, leaving the medulla, leads to hypoglycæmia and loss of liver glycogen. The condition can be remedied by injection of a steroid fraction, "cortin," obtained from cortical extracts. "Cortin" is a mixture of related steroids derived from androstane, several of which have a glucose-mobilising effect when injected into normal animals, as is revealed by hyperglycæmia, gain in liver glycogen, increase in glucose formation from amino acids, and retarded rate of glucose oxidation. "Cortin" injection does not necessarily lead to glycosuria, unless the animal is deficient in insulin, and unable to deal with the increased load of sugar.

Insulin.—Insulin, the chief hormone of the pancreas, controls carbohydrate metabolism by rendering the blood sugar more reactive, perhaps by promoting phosphorylation or subsequent degradation.

When insulin is injected into a normal animal the removal of glucose by the tissues is usually so rapid that the liver glycogen is broken down in an endeavour to maintain the glucose level in the blood.

The combination of both these effects results in a rapid fall in the blood sugar level, which may go below the minimal limit of 40–50 mg. per 100 ml., and induce *hypoglycæmia*, characterised by severe convulsions. These are rapidly relieved by glucose administration.

Muscle glycogen is not decreased under these conditions, showing that the essential function of this polysaccharide differs in the different tissues. Only hepatic glycogen can support directly the blood sugar concentration.

Severe experimental diabetes can be invoked by intramuscular injection of *alloxan*, which destroys the islet tissue of the pancreas (Shaw Dunn, 1943).

Pituitary Factor.—In 1930, Houssay showed that removal of the pituitary gland antagonised the glycosuria and ketogenesis in depancreatized dogs; and Burn and Ling showed that injection of extracts of the anterior pituitary was able to evoke ketogenesis in normal animals. From this it was concluded that the gland had a stimulating effect on fat metabolism which might lead to ketone formation when inadequately balanced by carbohydrate utilisation. That the effect is more complex is shown by the results of hypophysectomy in otherwise normal animals. In general, it appears to depress the secretion of sugar by the liver. Hypophysec-

tomised animals readily develop hypoglycaemia, and are very sensitive to carbohydrate starvation and to insulin injection. Conversely, adrenaline injection has very little effect in raising the blood sugar level, even when the hepatic glycogen store is ample, which suggests that the pituitary factor is necessary for hepatic glycogenolysis in dogs.

The hypoglycaemia due to phloridzin injections or starvation can be alleviated in hypophysectomised dogs by feeding with carbohydrate or protein, but not by feeding with fat, which has led Soskin (1935) to conclude that a pituitary factor promotes the conversion of fatty acids into sugar.

The combined effects of loss of both pancreas and anterior pituitary gland are shown in Houssay animals, which may live as long as nine months without special treatment although in a state precariously poised between fatal hypoglycaemia and diabetes. The glycosuria varies between 0.5 and 10 gm. *per diem*, as compared with the 50–70 gm. excreted daily on a similar diet by a dog from which the pancreas alone has been removed. Ketonuria is slight, and the glycogen levels in liver and muscle are normal. These remarkable phenomena indicate that the Houssay animal is able to utilise carbohydrate in the complete absence of pancreatic insulin.

The anterior pituitary gland is concerned largely in the co-ordination of the other ductless glands, and its complex influence on sugar metabolism is ascribed to the action of separate *hormotropic* factors, which evoke or repress the secretion of the adrenal cortex (adrenotropic effect), the pancreas (pancreotropic and glycotropic effect), the liver (hepatotropic and ketogenic effect), and the thyroid (thyrotropic effect).

Glycosuria.—The appearance of glucose in the urine, beyond the trace normally present, may be due to lowering of the renal threshold either congenitally (renal glycosuria) or experimentally (phloridzin glycosuria), or may be due to metabolic conditions which evoke hyperglycaemia. These include: (i.) alimentary glycosuria, which is rare in normal subjects; (ii.) insulin deficiency due to removal or disease of the pancreas (pancreatic diabetes); (iii.) excessive hepatic glycogenolysis, which may follow injection of adrenaline, pituitary extract, thyroxine, or may arise from the release of these hormones in asphyxia, general anaesthesia, emotional stress, toxic goitre, acromegaly, and experimental injury to the floor of the fourth ventricle (puncture diabetes).

The clinical condition, diabetes mellitus, is ascribed to pancreatic dysfunction leading to insulin deficiency, the glycosuria being due either to subnormal utilisation of glucose, or over-production of

glucose. In alloxan-evoked diabetes, the chief defect is the inability of the animal to use glucose for the synthesis of fatty acids (Stetten and Boxer, 1944).

Glucose tolerance expresses either (a) the total amount of carbohydrate that can be consumed daily without evoking glycosuria, or, more exactly (b) the dosage of glucose necessary to raise the blood sugar above the renal threshold. Alimentary glycosuria from carbohydrate over-feeding is very unusual, as the resources of the organism for carbohydrate storage and conversion are considerable.

In some pathological conditions, notably *diabetes mellitus*, glucose tolerance is low, and excess of carbohydrate in the diet readily evokes glycosuria.

Hypoglycaemia.—When the glucose content of the blood falls below a critical value, 0·07–0·03 per cent. in man, a characteristic hypoglycaemic syndrome sets in, the signs and symptoms of which are : (i.) extreme hunger, (ii.) fatigue and prostration, (iii.) motiveless anxiety, (iv.) tremors, (v.) vaso-motor unbalance with flushing or pallor, (vi.) delirium, coma, loss of deep reflexes. Hypoglycaemia has little effect on the contraction or irritability of the denervated muscle, and it is inferred that the motor disturbances arise centrally from glucose starvation of the nervous system. Hypoglycaemia may be evoked by injection of insulin, and abolished by injection of (i.) glucose, (ii.) adrenaline, which promotes hepatic glycogenolysis, and by (iii.) anterior pituitary extract, which antagonises insulin.

Experimental removal of the liver results in a rapid fall in blood sugar. Hepatectomised dogs require glucose to be infused at 0·25 gm. per kg. body weight per hour if hypoglycaemia is to be averted. This indicates that the liver of a 10 kg. dog during carbohydrate starvation is secreting about 64 gm. of glucose in twenty-four hours ; and, if the human body be comparable in efficiency, man's liver is producing up to 400 gm. of glucose *per diem*, an amount but little below the carbohydrate content of the ordinary diet.

In the absence of the liver, sugar disappears from the blood stream, which indicates the liver is concerned in the conversion of nutrients into glucose. Possibly, this mechanism is controlled by insulin, and the hyperglycaemia of diabetes is due to hepatic sugar production, unchecked owing to lack of insulin. Himsworth, however, presents evidence that in diabetes the tissues use less glucose than normally, which should relieve the demand on the liver. The high level of blood sugar, according to Himsworth, is an adjustment to provide the tissues with a bigger working load. When this exceeds the renal threshold level, sugar is lost, and the

liver must increase its output to maintain the sugar concentration under unfavourable conditions. The hepatotropic factor concerned in stimulating *neo-glucogenesis*, or formation of "new" glucose from sources other than glycogen, may be associated with anterior pituitary activity.

Sources of Blood Sugar

(1) *Carbohydrates*.—While it is believed that all, or almost all, of the blood sugar normally comes from hepatic glycogen, substances other than the simple hexoses can contribute either to glycogen or to sugar formation. The simplest of these are (+)-lactic acid (sarcolactic acid) and glycerol. Lactic acid is the characteristic end-product in muscle metabolism, and may be produced in other tissues during glycolysis. Glycerol is a constituent of all fats and complex lipides, and is liberated during digestion. The relative efficiency of these substrates has been computed by the Coris, who measured the glycogen increase in the livers of rats, previously starved for twenty-four to forty-eight hours, until the hepatic glycogen had fallen to 0.1–0.2 per cent.

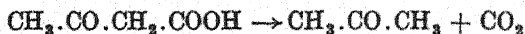
Food Material.	Absorption Time.	Amount absorbed per 100-gm. Body Weight.	Liver Glycogen.
Glucose . . .	4 hours.	1.06 gm.	5.3 per cent.
Fructose . . .	4 "	0.54 "	5.7 "
Galactose . . .	4 "	1.10 "	1.2 "
(+)-Lactic acid . .	3.5 "	0.11 "	1.2 "
Glycerol . . .	4 "	—	2.4 "

(2) *Proteins*.—Animals on a carbohydrate-free diet continue to store liver glycogen, showing that it can be obtained from sources other than food saccharides. Of these, the most obvious are the surplus amino acid residues after removal of the nitrogen. In diabetic animals unable to utilise sugar, the food or tissue proteins are diverted to the manufacture of the glucose which appears in the urine. In these conditions the "dextrose": nitrogen output in the urine, or D/N ratio, is about 2.8:1. In animals unable to retain sugar owing to phloridzin injections, the D/N ratio in the urine is as high as 3.6:1. Since 100 gm. of food protein yield about 16 gm. of urinary nitrogen, the value of the D/N ratio indicates that, in favourable circumstances, up to 58 per cent. of protein can be transformed into glycogen or glucose. By individual feeding experiments on phloridzin-treated animals it has been shown that the glucogenic amino acids are arginine, proline, hydroxyproline,

cystine, serine, alanine, glycine, glutamic acid, hydroxyglutamic acid and aspartic acid.

(3) *Fats*.—Addition of fat to the diet of a depancreatized or otherwise diabetic animal neither increases liver glycogen nor sugar excretion, and it was formerly assumed that carbohydrate cannot arise from fat in the animal body, apart from 10 per cent. of the molecule, which is released as glycerol. In pancreatic diabetes, however, the respiratory quotient may fall as low as 0.7, which indicates that fat alone is being oxidised.

Oxidation of sugars and of fatty acids in the animal is competitive, the preference being for the sugars. After a meal rich in carbohydrate, sugar oxidation dominates metabolism for several hours. In conditions of carbohydrate scarcity, fatty acid oxidation predominates, the depot fats being mobilised in the liver, and degraded to acetoacetic acid, pyruvic acid, acetic acid, and possibly other metabolites, all of which are released into the blood stream, and utilised by the peripheral tissues. If the carbohydrate shortage is acute, or if the blood sugar is unavailable owing to lack of insulin, fatty acid metabolism reaches an intensity beyond the capacity of the peripheral tissues for utilising fatty acid degradation products and acetoacetic acid accumulates in the blood and other tissues and may be decarboxylated to acetone :

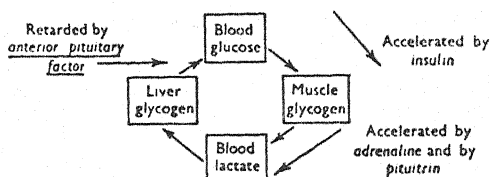


Acetone is relatively stable to oxidation in the animal, and can only be eliminated by the kidneys and the lungs.

Acetonaemia, or pathological concentration of acetone in the blood, results in the toxic condition of *ketosis*, or ketone-poisoning, characterised by drowsiness, and coma, leading to death. The condition is rapidly abolished by glucose and insulin administration, which repress the excessive acetoacetic acid production in the liver. Contrary to what was once believed, sugars have no *ketolytic*, or ketone-destroying, effect; their action is anti-ketogenic in that they provide a more acceptable source of energy than the fats (Koehler *et al.*, 1941).

The Glucose-lactate Cycle.—Lactate is a constant constituent of human blood, the range being 5–20 mg. per 100 ml. in the resting subject. Moderate exercise may raise the value to 40 or 50 mg., while violent exercise has raised it as high as 200 mg. (A. V. Hill, 1924).

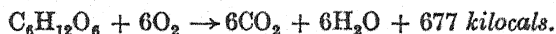
A fraction of this lactate is excreted by the urine, a fraction may undergo oxidation in the tissues, but the bulk of it, in rats, as Cori and Cori have shown, is rebuilt into glycogen in the liver. Thus, a sugar molecule can traverse a complete cycle in the organism.



The glucose-lactate cycle. (Cori and Cori.)

At the start of exercise, there is a rise in the blood-lactate level in dogs. This soon falls, and does not rise again, unless exercise be prolonged and severe, for lactate does not easily escape from muscle, except in states of inadequate oxygen supply. Hence, Cherry and Crandall (1939) conclude that the glucose-lactate cycle involving muscle and liver does not operate significantly during ordinary activity.

The Heat Value and Respiratory Quotient of Carbohydrates.— Each gram molecule of hexose completely oxidised in the body requires six gm. mols. of oxygen, and forms six gm. mols. of carbon dioxide, the process being accompanied by the liberation of 677 kilocalories of energy as heat.



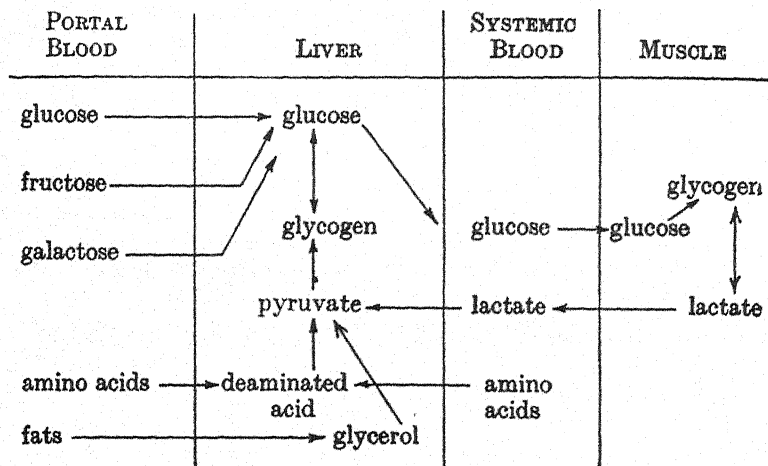
Expressed otherwise, the heat value of a hexose is approximately 3.77 kilocalories per gram, the value being higher (4.2) for the polysaccharides, which are less hydrated. Also, since the volume of oxygen consumed equals the volume of carbon dioxide produced, the respiratory quotient is 1, where *R.Q.* (the quotient) is expressed as CO_2/O_2 .

When the respiratory quotient of an organism or a tissue is unity, the inference is drawn that carbohydrate, or possibly lactate is the substance oxidised.

Oxygen and Carbohydrate consumed in Walking One Mile at Different Speeds (A. V. Hill, 1933)

Speed, in Miles an Hour.	Heat Liberated, in Kilocalories.	Rise of Body Temperature.	Oxygen consumed, litres.	Sugar Oxidised grams.
2	30	0.5° C.	6	8
3.5	60	1	12	16
5	115	2	23	31

Carbohydrate Circulation in the Animal Body



CARBOHYDRATE UTILISATION

Carbohydrates, especially glucose, provide an important source of energy for living cells. This energy is obtained in two different ways: (i.) In the absence of free oxygen, by fermentation, or glycolytic degradation of the sugar into simpler compounds with a lower energy value, and (ii.) in the presence of oxygen, by respiration, or oxidation.

(i.) **Anaerobic glycolysis**, or fermentation, appears to be a primitive endowment of all cells, and is characteristic of the life of lower plants, notably yeasts and bacteria. The process is uneconomical in that the end-products are lactate, alcohol or similar combustible compounds, the energy of which is unavailable for the organism. In higher animals, anaerobic glycolysis is restricted almost entirely to the muscular system, where it forms part of the highly complex and specialised mechanism of contraction. Malignant tissues obtain energy by anaerobic glycolysis, which enables them to grow rapidly in circumstances of restricted oxygen supply.

The Pasteur Effect.—Fermentation is characterised by a high rate of carbohydrate destruction and an accumulation of incompletely oxidised end-products. It is antagonised by free oxygen, which preserves the carbohydrates of the cell from extravagant wastage, and restricts the products of anaerobic metabolism. This inhibition of fermentation by oxygen is termed the Pasteur effect, after its discoverer, who showed that the ratio, *weight of sugar*

destroyed to weight of yeast formed, during alcoholic fermentation, varied from 176 : 1 (in complete absence of air) to 4 : 1 (in presence of excess of oxygen).

(ii.) **Aerobic oxidation**, or respiration, takes place in presence of free oxygen, and results in the formation of the completely oxidised end-products carbon dioxide and water. Respiration is a fundamental property of all active tissues and is found in all organisms, with the notable exception of the obligatory anaerobes, which fail to grow when the oxygen-content of their environment has reached a value sufficient to inhibit completely their anaerobic metabolism.

(iii.) **Aerobic glycolysis**, or fermentation in presence of oxygen, is displayed by a few fungi, notably brewer's yeast. It does not occur in the majority of animal cells, apart from the production of small amounts of lactate by tissues with an exceptional ability for utilising sugar, such as brain cortex, mammalian retina, and embryonic structures. Cyanide and other respiratory poisons may induce or reveal aerobic glycolysis in tissues by inhibiting the entry of free oxygen into the respiration process. Nearly all malignant tissues exhibit marked aerobic glycolysis.

In the animal, carbohydrate traffic is dominated by the activities of five specialised tissues : (1) skeletal muscle, (2) cardiac muscle, (3) brain cortex, (4) kidney, and (5) liver.

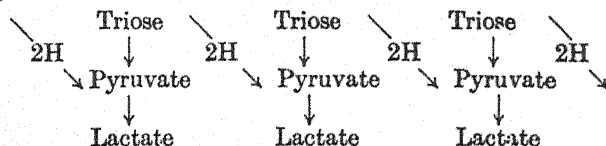
1. Skeletal Muscle

Muscle is an apparatus for the rapid conversion of chemical energy into heat and mechanical work. As regards rate and continuity, this proceeds uniformly in unstriated and cardiac muscle, but striated skeletal muscle is under voluntary control and subject to abrupt and occasional demands from the organism. Skeletal muscle, in consequence, requires a store of energy that can be released rapidly under anaerobic conditions, and replenished under aerobic conditions in the resting tissue.

The contraction process is accompanied by two physical events capable of exact measurement : heat liberation and mechanical work. The process is anaerobic in that it can continue for some considerable time in absence of oxygen, during which period glycogen is converted into lactate, the characteristic end-product of muscle activity.

In outline the change involves four stages in degradation : (a) glycogen to hexose, (b) hexose to two molecules of a C_3 sugar, or triose, (c) oxidation of triose to pyruvate, and (d) reduction of pyruvate to lactate. The energy required for the reaction is transferred by means of phosphoric acid units supplied by two carriers, phosphocreatine and adenosine triphosphate, and is catalysed by a system of at least ten enzymes.

The process forms a cyclic reaction chain in which pyruvate is reduced to lactate by accepting two atoms of hydrogen from a triose, which thereby becomes dehydrogenated to another molecule of pyruvate.



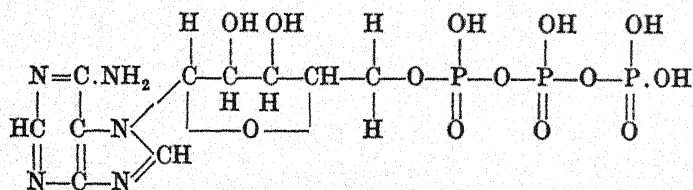
In order to react, the triose and the pyruvate are activated by specific dehydrogenases (p. 248), and intermediate phosphoric esters are formed. Three interlinked chemical systems are operated:

- (1) Adenosine triphosphate \longleftrightarrow adenylic acid + $2\text{H}_3\text{PO}_4$.
- (2) Phosphocreatine \longleftrightarrow creatine + H_3PO_4 .
- (3) Glycogen \longleftrightarrow hexose monophosphate \longleftrightarrow lactic acid.

The first two systems are independent of oxidation; the third is semi-anaerobic in that glycogen break-down (glycogenolysis) proceeds in absence of oxygen, whereas glycogen formation (glycogenesis) requires oxygen, and takes place during the recovery stage in muscle.

(1) **The Adenosine Triphosphate System.**—Fresh mammalian muscle contains about 0.01–0.02 per cent. of adenosine triphosphate, or adenylic pyrophosphate, a diphosphoric ester of adenylic acid which is a nucleotide assembled from adenine, D-ribose and phosphoric acid (p. 407).

Adenosine triphosphate (*ATP*) functions as a substrate for *actomyosin*, the contractile protein of muscle, which is reversibly dissociated into *actin* and *myosin* during the hydrolysis of *ATP* to adenosine diphosphate (*ADP*) and H_3PO_4 (Szent-Györgyi and Straub, 1945). When muscle contracts, this phosphate is transferred to the glycogen-lactate system. The *ATP* is recharged with phosphate as the muscle relaxes.

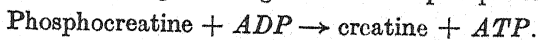


Adenosine Triphosphate, *ATP*.
(adenylic pyrophosphate).

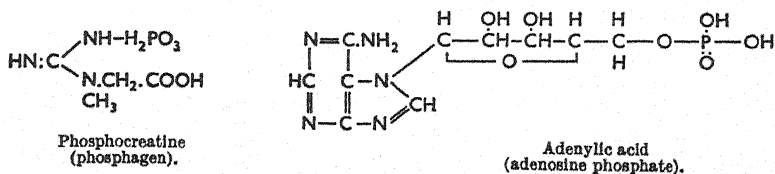
(2) **The Phosphocreatine System.**—Fresh muscle contains 0.5–0.6 per cent. of creatine, present as a non-diffusible phosphate

INTERMEDIATE METABOLISM: CARBOHYDRATES 333

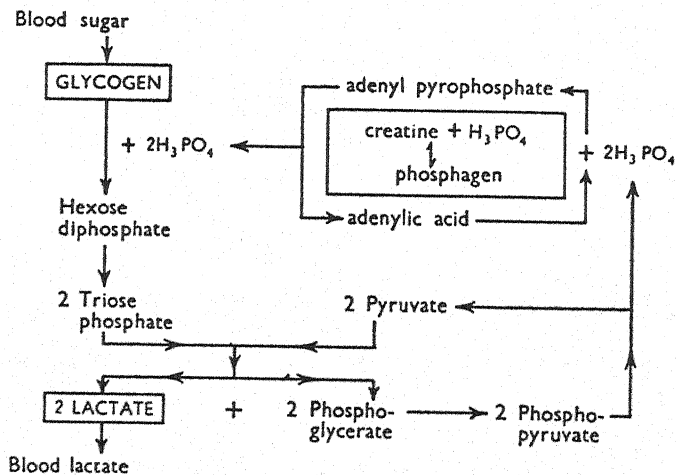
(*phosphagen*), which readily transfers phosphoric acid to adenosine diphosphate, thereby regenerating adenosine triphosphate.



The phosphagen is regenerated again at a later stage in the contraction process.



(3) The Glycogen-lactate System.



Cycle of Events during Muscle Contraction.

Stages in Muscle Glycogenolysis.—Resting muscle contains about 1 per cent. of glycogen, part of which is converted into (+) lactic acid (sarcoplactic acid) during the contraction process. This lactic acid disappears during the aerobic recovery process. In frogs it is reconverted into muscle glycogen, but in mammals some of it escapes into the blood stream and travels to the liver, where it is converted into hepatic glycogen. When the oxygen supply is adequate, lactic acid never exceeds a minimal value, both in resting and contracting muscle. In the absence of oxygen, lactic acid steadily accumulates, even in resting muscles, until the state of *rigor mortis* is reached.

The rate of acid formation is greatly increased by contraction

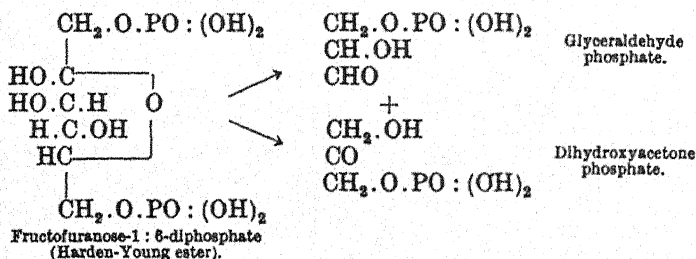
under anaerobic conditions, the amount of acid formed being related to the work done by the muscle.

These changes were first elucidated by Hopkins and Fletcher, who taught subsequent workers "to respect the biological qualities" of living tissues. By disintegrating the fresh muscle in ice-cold alcohol they were able to obtain extracts unchanged by catalysis during the process of analytical treatment.

Stage I.—Glycogen is phosphorylated and resolved into sugar units, $\text{glycogen} + \text{H}_3\text{PO}_4 \rightarrow \text{glucose-1-phosphate}$ (Cori ester, p. 111). *Glucose-1-phosphate* is transformed to *glucose-6-phosphate*, and phosphorylated to a hexose diphosphate, *fructose diphosphate*, the Harden-Young ester (p. 120).

Muscle extracts transform glycogen into lactic acid much more rapidly than they transform glucose into lactic acid, from which it is concluded that the polysaccharide is phosphorylated directly, and not first depolymerised to a hexose.

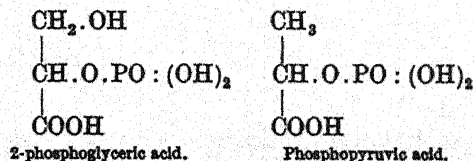
Stage II.—Fructose diphosphate splits into two molecules of triose phosphate: *dihydroxyacetone phosphate* and *glyceraldehyde phosphate*. The catalyst is *zymohexase*, a mixture of *aldolase*, which splits the hexose, and *isomerase*, which catalyses the equilibrium between the triose phosphates.



Stage III.—Two molecules of triose phosphate interact with two molecules of pyruvic acid to form lactic acid and 2-phosphoglyceric acid (α -phosphoglyceric acid).

The oxidation of the triose phosphate to phosphoglycerate is catalysed by a dehydrogenase working in conjunction with co-enzyme and adenylic acid.

Stage IV.—2-phosphoglyceric acid is converted to phosphopyruvic acid, thereby building-up phosphate-bond energy.



Stage V.—Phosphopyruvic acid reacts with adenylic acid to form adenosine triphosphate and pyruvic acid, $\text{CH}_3\text{CO}\cdot\text{COOH}$.

Stage VI.—Pyruvic acid is reduced to lactic acid,



by the triose phosphate formed in Stage II.

These changes constitute the Parnas cycle of muscle metabolism as revised by later workers, and are partially retraversed during the recovery process. They have been elucidated by comparison with similar changes taking place when sugar is fermented by yeast.

Glycogenolysis Inhibition.—Dialysis of muscle extracts deprives them of glycogenolytic power owing to removal of (i.) magnesium, which activates the muscle phosphatase concerned in phosphorylation of the carbohydrates; (ii.) adenosine triphosphate, the phosphate-carrying agent of the system. Active inhibition can be brought about by (i.) iodoacetic acid, which poisons the triose phosphate dehydrogenase, and by (ii.) sodium fluoride, which stops the resolution of the hexose diphosphate in Stage II. By a selective use of these specific inhibitors, the intermediate products of glycogenolysis have been concentrated and identified.

The Aerobic Process.—A resting skeletal muscle consumes about 6 cubic mm. of oxygen per gm.² of dry weight per hour. During active contraction the oxygen consumption rises to 40, or more. This additional oxygen is not required by the Parnas cycle, and its uptake is explained by Sacks (1941), who postulates the existence of three independent mechanisms in muscle: (1) anaerobic reversible conversion of glycogen to hexose phosphate, which operates in the first period of contraction; (2) aerobic conversion of glycogen to lactate, which begins and is maintained when the blood and oxygen supply to the muscle has increased in response to activity; and (3) anaerobic conversion of glycogen to lactate by the Parnas cycle, which only operates in overstrained, asphyxiated, or otherwise damaged muscle, and is not part of the normal contraction sequence.

2. Cardiac Muscle

Heart muscle differs from skeletal muscle in structure and in function. During the lifetime of the animal the heart never rests. Its activity is almost constant, and its recovery period is limited to the refractory phase between each beat. Its creatine content is only 0.2–0.3 per cent., or about half that of skeletal muscle.

Heart muscle contains 0.1–0.2 per cent. of glycogen, which

under anaerobic conditions is transformed into lactate, showing that the heart possesses a metabolic equipment resembling that of skeletal muscle. Apart from this, the two tissues differ in fundamental respects. The heart has a respiratory quotient between 0.8 and 0.9, which signifies that either protein or a mixture of fat and carbohydrate are the food materials oxidised. Under the aerobic conditions of normal working, the glycogen content of the heart appears to be constant, and remains constant as long as the heart is supplied with glucose.

This is also observed if the glycogen-lactate cycle be stopped by iodoacetic acid. Under anaerobic conditions, such as those of asphyxia, the heart can continue to beat provided the lactic acid is neutralised as rapidly as it is formed. Unlike skeletal muscle, the heart is very sensitive to lactic acid, and responds immediately by a protective dilatation of the coronary arteries. Unless the perfusion fluid be kept decidedly alkaline (pH 8.0), the oxygen-starved heart stops beating within a few minutes, owing to acid inhibition. When the perfusion fluid is adequately buffered the heart can continue to beat under anaerobic conditions, its source of energy being obtainable from glucose, probably by way of neutral lactate.

These observations show that the heart is endowed with :—

(i.) An aerobic metabolism working under normal conditions, and probably involving an adenine triphosphate + phosphagen mechanism similar to that in skeletal muscle, but regenerated by a different metabolic cycle.

(ii.) An anaerobic metabolism similar to the Parnas cycle, which comes into operation in conditions of oxygen deficiency, and can continue to work as long as the pH of the blood is kept on the alkaline side of 7.5.

3. Brain Cortex

Man's body has two wardens. The autonomic nervous system and its associated endocrine glands determine the unconscious life and personality; the cerebral cortex and its sensory and motor tracts form the apparatus of voluntary expression. Both structures are composed of nerve cells and their extensions and supporting tissues.

The study of the metabolism of nervous tissue is confronted by a problem that is unique and apparently of insuperable difficulty.

In every other active tissue and organ of the body, metabolism and function can be correlated. But the brain, silent and motionless, traffics with the imponderable. That the brain requires an abundance of oxygen is inferred from the elaborate arterial supply,

and the fact that an acute anoxæmia may be sufficient to bring about loss of consciousness in less than a minute. Indeed, as Barcroft has observed, it is strange that the most important tissue in the body should have no oxygen reserves. By a comparison of the O_2 and CO_2 content of the blood entering and leaving the skull cavity, it has been estimated that the oxygen consumption of the dog's brain is approximately 0.14 ml. O_2 per gram of fresh tissue, per minute, a value much higher than that calculated from the oxygen consumption of cortical tissue in the manometer.

That the brain requires glucose for its maintenance is suggested by the disturbances of the central nervous system—*anxiety*, *confusion*, *delirium* and *convulsions*—that constitute the hypoglycæmic syndrome evoked by insulin, sometimes applied, empirically, for the treatment of schizophrenia (*dementia præcox*). Under experimental conditions, brain tissue suspended in saline is able to oxidise a variety of substrates, including glucose, mannose, fructose, glutamate, succinate, glycerophosphate, pyruvate and lactate, but of all these, only glucose and mannose are able to relieve immediately the signs and symptoms of hypoglycæmia, when injected into the depleted animal. For fructose to be effective it requires to be converted into its isomers by the liver.

By comparative analysis of arterial and internal jugular blood, Gibbs (1942) found that, under conditions of average flow, 10 mg. of sugar are removed from every 100 ml. of blood passing through the human brain. The increase in CO_2 and decrease in O_2 indicate an R.Q. of 1, showing that the chief source of energy for brain metabolism is glucose, most of which is completely oxidised, as very little reappears as lactate in the venous blood.

Narcotics, in concentrations capable of anæsthetising the living animal, are able reversibly to inhibit oxygen-uptake by brain slices. The effect is greatest when the substrate is glucose, and least when it is succinate (Quastel).

Under aerobic conditions, glucose oxidation by the cortex produces very little lactic acid. Under anaerobic conditions glycolysis sets in, and lactic acid is produced with great rapidity. Brain cortex shows a typical Pasteur effect, in that the quantity of glucose degraded anaerobically is relatively greater than the quantity oxidised under aerobic conditions.

Kendal Dixon (1937) has shown that the addition of potassium salts in high concentration (M/10) to the aerobic system results in a considerable rise in oxygen consumption and a great increase in lactate formation. This reversal of the Pasteur effect is ascribed to an alteration in cell permeability by the K^+ ions.

Avitaminosis B₁.—The acute signs of vitamin B_1 deficiency in birds, *opisthotonus* (retraction of the head), failure of vision, loss

of temperature control, convulsions, have been traced to the accumulation of lactate and pyruvate in the lower part of the brain (Peters, 1936).

Lactate is converted to pyruvate by lactate dehydrogenase, and the pyruvate subsequently undergoes oxidation to simpler products.

When an animal is killed by acute asphyxia, or by cutting off the blood supply to the brain, the brain potential waves, as recorded electrically by the *encephalograph*, stop in a few seconds, which suggests that they originate from the aerobic respiration of brain tissue. If an animal is killed by injection sufficient of Ca^{++} , Mg^{++} and K^+ , the brain potential waves may continue for 15 minutes after "death" (Rubin *et al.*, 1942).

Vitamin B_1 provides the co-enzyme necessary for the pyruvate oxidation. Avitaminous brain tissue has a lowered rate of oxygen consumption, which Peters has shown may be restored to the normal value by addition of vitamin B_1 .

Summary of the History of Muscle Biochemistry

1903.—Buchner obtained from yeast a cell-free extract, *zymase*, capable of catalysing the breakdown of glucose to alcohol and carbon dioxide.

1906.—Harden and Young showed that *zymase* required phosphate and a co-enzyme, *co-zymase*, in order to act.

1912.—Harden obtained hexosephosphates from fermenting sugars, and showed that phosphorylation is the first stage in *zymolysis*.

1912.—Emden obtained a cell-free extract from muscle capable of forming lactic acid from hexosediphosphate, *lactacidogen*. Later, he found that normal muscle contains no hexosediphosphate, but only a monophosphate, which is subsequently converted into diphosphate.

1913.—Dakin and Dudley, and Neuberg, independently discovered *glyoxalase*, a widely-distributed tissue enzyme capable of converting methyl glyoxal (or other ketonic aldehydes) into lactic acid (or a corresponding hydroxy acid). Hence, it was suggested that methyl glyoxal is the immediate precursor of lactic acid in muscle metabolism.

1926.—Phosphocreatine discovered in muscle by the Eggletons.

1926.—Meyerhof obtained *glycogenase* from muscle, and showed that it required phosphate and a co-enzyme in order to be able to catalyse the conversion of glycogen to lactic acid.

1928.—Adenosine triphosphate discovered in muscle by Lohmann.

1932.—Lohmann showed that *glyoxalase* requires a co-enzyme, namely glutathione. However, if muscle extract is freed from

glutathione it can still transform glycogen, but not methyl glyoxal to lactic acid. This transformation requires the addition of Mg ions and adenosine triphosphate, which were removed by the dialysis. If only glutathione be added, then methyl glyoxal, but not glycogen, is transformed into lactic acid. Hence, it was concluded that the main path for the conversion of lactacidogens to lactic acid is not by way of methyl glyoxal.

1933.—Embden showed that fluoride inhibits formation of lactic acid from glycogen in muscle tissue, and leads to an accumulation of *phosphoglyceric acid*, derived from the hexosediphosphate. Phosphoglyceric acid is readily transformed to pyruvic and phosphoric acids by muscle enzymes in the absence of fluoride. From these data Embden suggested a cycle, involving four stages: (i.) resolution of hexosediphosphate into two molecules of *triose phosphate* (glyceraldehyde phosphate and dihydroxyacetone phosphate); (ii.) dismutation of the triose phosphates into glycerophosphate and phosphoglyceric acid; (iii.) resolution of phosphoglyceric acid into pyruvic and phosphoric acids; (iv.) interaction between pyruvic and glycerophosphoric acids to form lactic acid and a triose phosphate (glyceraldehyde phosphate), which rejoins the cycle at Stage (ii.).

1933.—Meyerhof confirmed and extended Embden's observations by showing that pyruvate accumulates in muscle poisoned by sulphite, and is derived from the hexosediphosphate, and not, as was suggested, from lactic acid by dehydrogenation. Three muscle enzymes were separated: *aldolase*, which catalyses the conversion of hexosediphosphate into triosephosphates; *phosphoglyceromutase*, and *enolase*, which catalyse the chain reaction whereby phosphoglyceric acid gives rise to pyruvic acid.

1934.—Lundsgaard showed that muscle poisoned by iodoacetic acid is able to contract for a short time in an atmosphere of nitrogen without producing lactic acid, the energy being derived from the breakdown of phosphagen (phosphocreatine). Hexosediphosphate and triosephosphates accumulate, but are unable to undergo further change.

1934.—Parnas showed that in muscle extracts glycogen reacts with inorganic phosphate to form a hexosemonophosphate, now identified as Cori's ester, from which the diphosphate is subsequently derived.

1934.—Jost showed that anaerobic breakdown of carbohydrate to lactic acid in kidney tissue proceeds on the same lines as in muscle.

A similar type of cycle was demonstrated in brain (Euler, 1936), and in heart muscle (Ochoa, 1937).

1939.—Engelhardt and Lyubimova discovered that myosin, the chief protein of the muscle fibre, catalyses dephosphorylation of adenosine triphosphate, and acquires energy for contraction.

1941.—Sacks described an aerobic contraction mechanism independent of the Meyerhof-Parnas cycle.

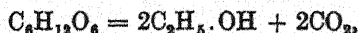
1945.—Szent-Györgyi and Straub isolated in crystalline form the enzyme, *actomyosin*, which dissociates into myosin and actin, thus forming the contractile system of the muscle.

Sugar Fermentation by Yeast

Natural sugars are liable to attack by yeasts and bacteria, and undergo either aerobic oxidation or anaerobic fermentation, according to circumstances, which led Pasteur to describe fermentation as "life without air." Brewer's yeast (a culture yeast of the species *Saccharomyces cerevisiae*) and some facultatively anaerobic bacteria (the lactic and propionic bacilli), however, are able to ferment sugars when oxygen is present, and for this reason are employed industrially in the production of alcohol and lactic acid.

The term *fermentation* (L. *fervere*, to boil) was originally applied to the breakdown of sugar by yeast, on account of the liberation of gaseous carbon dioxide, which caused the mixture to froth. Subsequently, the term was applied to any non-putrefactive change brought about by the growth of living organisms. Other common fermentations include: the *lactic fermentation*, or souring of milk, in which lactic acid is formed from lactose; the *acetic fermentation*, or souring of wine, owing to conversion of ethyl alcohol into acetic acid; and the *ammoniacal fermentation* of urine, in which urea is converted into ammonium carbonate. Yeasts are found naturally wherever sugar is available. They grow less rapidly than bacteria, but can flourish in material preserved from bacterial attack by its high acidity or high osmotic pressure. By forming alcohol, yeasts can sterilise their media to moulds and bacteria. "Top" fermentation yeasts tend to cluster and to produce CO₂ vigorously. Bread-making yeast is a "top" strain.

Alcoholic fermentation of sugar is represented by the outline equation,

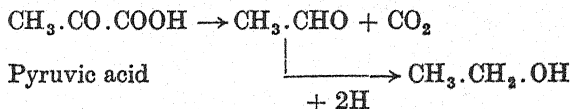


in which the monosaccharide represents one of the four fermentable hexoses, D-glucose, D-mannose, D-fructose and D-galactose. The last of these is only attacked by yeasts previously conditioned by being cultivated in a medium containing galactose.

Sugar fermentation follows the same path as muscle glycolysis in that a chain of intermediate phosphate esters is formed; but when the pyruvate stage is reached, the enzyme *pyruvic carboxylase*, which is present in yeast but not in muscle, decarboxylates the

pyruvic acid to *acetaldehyde* and *carbon dioxide*, a characteristic end-product of the fermentation.

The acetaldehyde is subsequently reduced to ethyl alcohol.



Factors in Sugar Fermentation.—(1) *Zymase*.—Cell-free extracts from yeast are able to ferment sugars in absence of living organisms. This property was ascribed to an enzyme complex *zymase*, present in the extract.

Harden and Young later showed that *zymase* solutions were inactivated by dialysis owing to the loss of two indispensable coactants, both of which were heat-stable. The first of these proved to be inorganic phosphate, the second was an organic compound, which Harden called "the co-ferment of alcoholic fermentation." This organic factor, renamed *co-zymase*, or co-enzyme I, was identified by Euler (p. 288). It is easily extracted from yeast by washing with water; the residual mixture of enzymes and activators has been termed *apo-zymase*. The function of co-zymase is to transfer phosphoric acid to the sugar substrates during the fermentation.

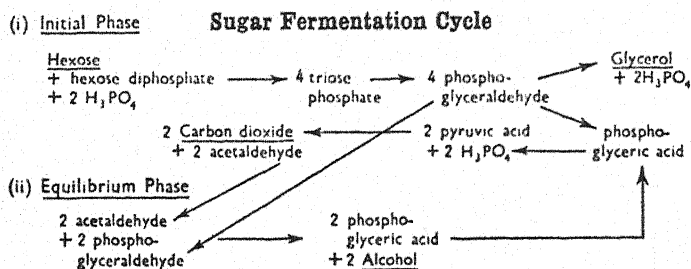
(2) *Pyruvic Acid and Acetaldehyde*.—The optimal pH for sugar fermentation by living yeast is about 5-6. If the mixture be kept on the alkaline side of neutrality there is a steady accumulation of pyruvate instead of alcohol. Similarly, if sodium hydrogen sulphite (bisulphite) be added to the mixture, there is an accumulation of acetaldehyde, which is bound by union with the sulphite. Since pyruvic acid is transformed into acetaldehyde by *pyruvic carboxylase*, and since acetaldehyde when added to the fermenting mixture is hydrogenated to alcohol, it is concluded that these two compounds are successive intermediates in sugar fermentation.

(3) *Sugar Phosphates*.—Before the sugar is attacked it is converted into an unstable form by the enzyme *hexokinase*, which is present in yeast but not in muscle. Phosphorylation accompanies this process, and the hexose phosphate is resolved into two molecules of triose phosphate.

(4) *Glycerol* is an invariable by-product of sugar fermentation. It appears in the early stages of the process, and reaches a maximum concentration which does not change appreciably during prolonged glycolysis. However, if fermentation be checked by the addition of sulphite, which does not allow it to proceed beyond the aldehyde stage, there is a steady increase in the production of glycerol.

Under these conditions the end-products of sugar fermentation by yeast are glycerol, acetaldehyde and carbon dioxide, a consequence which proved of national, though not international, value in providing the central European countries with glycerol for explosive manufacture during war periods.

The early formation of glycerol is explained by the dual nature of the fermentation process, which consists of: (i.) an initial phase before aldehyde has been formed, and (ii.) an equilibrium phase when aldehyde is interacting with the glycerol precursor, phosphoglyceraldehyde. These phases have been elucidated largely by the work of Embden, Meyerhof and Kieserling.



Meyerhof has shown that hexose phosphorylation requires Mg ions and co-enzyme, which reacts with the sugar to yield hexose diphosphate and adenylic acid in a manner comparable to the action of adenine triphosphate as a phosphate carrier in muscle glycolysis.

CARBOHYDRATE SYNTHESIS BY PLANTS

The assimilation of CO₂ by a carboxylation process in both plants and animals was first established by Wood and Werkman (1935), and provides an explanation of the long-disputed mechanism for the photosynthesis of carbohydrates by green plants. The first stage is the fixation of CO₂ by an aldehyde or other acceptor. This stage occurs both in animals and plants, and is not dependent on light. The second stage, which is restricted to green plants and a few pigmented algae and micro-organisms, is a photochemical reduction of the fixed CO₂ to the primitive sugar unit, possibly a triose, which, in turn, may fix another molecule of CO₂, and undergo further reduction. Using an atmosphere containing 1-5 per cent. of CO₂, labelled by inclusion of radioactive carbon, ¹⁴C, Ruben (1940) has shown that CO₂ fixation in green plants can occur in the dark, and is not dependent on the chlorophyll concentration. The isotope reappears as a carboxyl group, —¹⁴COOH. In the second stage,

which requires both chlorophyll and light, less CO_2 reappears as $-\text{COOH}$, but can be traced to a fraction with a sedimentation rate corresponding to a molecular weight of about 350.

Fixation $\text{R.H} + \text{CO}_2 \rightarrow \text{R.COOH}$ *Dark reaction.*

Reduction $\text{R.COOH} + \text{H}_2\text{O} \rightarrow \text{R.CH}_2.\text{OH} + \text{O}_2$ *Light reaction.*

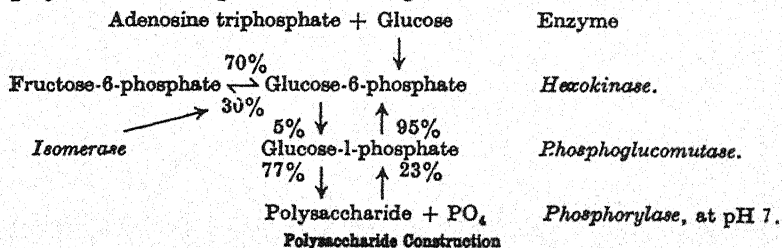
Fixation $\text{R.CH}_2.\text{OH} + \text{CO}_2 \rightarrow \text{R.CH(OH).COOH} \rightarrow \text{Carbohydrate.}$

There is no evidence that the assimilated CO_2 is converted into any volatile compound; such as formaldehyde, CH_2O , which has never been shown unmistakably to occur in plant metabolism, although it may be formed when CO_2 and water-vapour are subjected to prolonged ultra-violet irradiation in presence of catalysts (Baly, 1940).

For photo-reduction of fixed CO_2 , the chlorophyll pigment system of the leaf must be intact.

POLYSACCHARIDE CONSTRUCTION IN PLANTS AND ANIMALS

The common pathway for starch or glycogen construction is by glucose-6-phosphate, which can arise from phosphopyruvic acid, by a reversal of the Embden-Meyerhof cycle, or can be formed from adenosine triphosphate and glucose, the phosphorylation being catalysed by hexokinase. Glucose-6-phosphate reverts to glucose-1-phosphate, when activated by phosphogluco-mutase, and reverts to fructose-6-phosphate, when activated by isomerase. From glucose-1-phosphate, activated by potato phosphorylase, Hanes (1940) has been able to synthesise a starch showing the same X-ray diffraction pattern and amylose-iodine reaction as native potato starch. While, using animal phosphorylase from liver, heart or brain, Cori and others have synthesised glycogen from glucose-1-phosphate. Muscle phosphorylase, obtained in crystalline form by Green, Cori and Cori (1943), yields an unbranched polysaccharide resembling starch rather than glycogen, in that it gives a blue iodine reaction. If a supplementary enzyme, obtainable from heart or liver, be present, a polysaccharide branched like natural glycogen is formed. In presence of the appropriate phosphatases, the various sugar phosphates exist as an equilibrium system, capable of providing polysaccharide or glucose according to circumstances.



Lactose Formation in the Animal Body.—Lactose, the characteristic disaccharide of milk, is a foreign sugar within the organism, and when injected into the circulation is excreted by the kidney, a phenomenon seen in the overflow lactosuria that accompanies active lactation. This indicates that the sugar must be assembled locally in the mammary gland and secreted directly in the milk. The amount of glucose removed from the blood by the lactating gland depends on the arterial level of blood sugar, which is relatively constant for the species.

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CHAPTER 17

INTERMEDIATE METABOLISM: PROTEINS

DURING digestion amino acids are liberated from the hydrolysed food proteins and absorbed into the portal blood. The effective surface of the small intestine is enormously increased by the presence of the villi, and represents 8–10 sq. metres in the adult human subject. Amino acid absorption is ascribed to simple diffusion, and owing to the relative slowness of protein digestion, proceeds at about 1 gm. per kg. of body weight per hour (Pflüger).

It is possible that some of the smaller peptides are also absorbed. The amino acid content of human plasma is expressed in terms of amino nitrogen, measurement of each individual amino acid being impracticable in small samples. In the resting subject, plasma amino nitrogen is about 3 mg. per 100 ml. Values between 5.2 and 7.2 mg. have been reported for entire blood. Ingestion of protein foods results in a rise in the amino acid values within two hours of the meal, and reaches a maximum about four hours after the beginning of the meal. Circulating amino acids may be stored temporarily in the tissues during the absorption peak, and the concentration of tissue amino acids may rise considerably above the blood value maximum of about 10 mg. amino N per 100 ml.

General History of the Amino Acids.—After absorption and circulation, amino acids may undergo :—

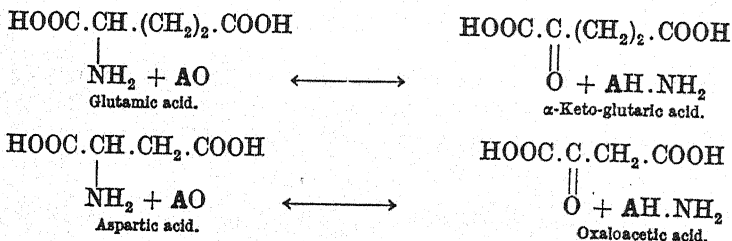
- (i.) Temporary storage in muscle, liver, kidney and other tissues.
- (ii.) Conversion into closely related derivatives.
- (iii.) Elaboration into tissue proteins.
- (iv.) Trans-amination, or exchange of amino groups.
- (v.) Trans-methylation, or transfer of $-\text{CH}_3$ groups.
- (vi.) Deamination, or degradation by loss of the α -amino group.

As trans-amination and deamination are common events in the history of amino acids, they will be considered first.

Trans-amination.—Outside the organism, amino acids are relatively stable compounds; within the organism, the α -amino group becomes very reactive, and may be transferred to an amino acceptor, or liberated as ammonia by the agency of suitable catalysts. Amino transference was first observed by Braunstein and Kritzmann (1937), who named it "trans-amination." It is a reversible process, and is catalysed by the enzymes *glutamic*

and *aspartic dehydrogenases* or *aminopherases*, which occur in muscle, liver, and many other tissues.

Trans-amination requires a specific amino donor, glutamic, or aspartic acid, or a specific amino acceptor, α -keto-glutaric acid, or oxaloacetic acid, according to the direction of the reaction.

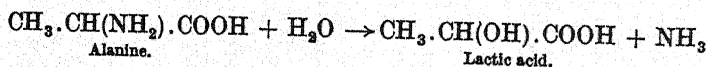


AO represents a specific amino acceptor, such as an α -keto acid. In the reverse reaction, AH.NH₂ represents a suitable amino donor, which may be one of the natural amino acids. That is to say, the aminopherase system, working in one direction, can convert monocarboxy keto acids such as pyruvic, into amino acids by donation of —NH₂; and working in the reverse direction, aminopherases can deaminate amino acids to keto acids by removal of —NH₂. Such systems are of obvious importance in the natural synthesis of amino acids from carbohydrate residues and products.

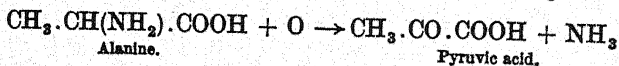
By feeding tyrosine containing the nitrogen isotope, ¹⁵N, to normal rats, Schoenheimer has found that isotopic tyrosine can be incorporated into tissue proteins, and furthermore, that the isotope ¹⁵N can be detected in other amino acids, showing that an exchange of amino nitrogen is continuously taking place (1942).

Deamination.—Amino acids can lose an α -amino group hydrolytically in two ways :—

(a) Direct hydrolysis to the corresponding hydroxy acid,



(b) Dehydrogenation (oxidation) to the corresponding keto acid,

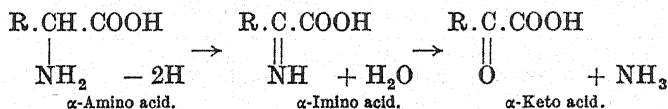


The frequent appearance of α -hydroxy acids in plant and animal tissues suggests that hydrolytic deamination is a biological process, and this is supported by the work of Weitzmann and Bergmann (1938), who have obtained the corresponding hydroxy

acids by the action of ultra-violet light on aqueous solutions of amino acids.

Oxidative deamination, however, is a well-established process. It can be brought about by the action of hydrogen peroxide, or by deaminating enzymes (amino acid dehydrogenases) present in certain tissues, and the resulting keto acids have been isolated and identified (Krebs, Bernheim).

The mechanism appears to involve: (i.) dehydrogenation of the amino group to an imino group; and (ii.) hydrolysis of the imino compound to a keto acid and ammonia.



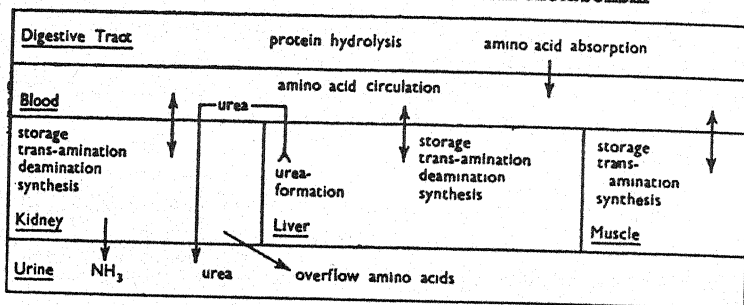
Deaminating enzymes occur in kidney cortex and, to a lesser extent, in liver. Vertebrate intestinal mucosa and cardiac muscle of pigeons and frogs have a slight and restricted capacity for deamination. Other vertebrate tissues (brain, retina, spleen, bone-marrow, pancreas, salivary gland and mammalian heart) appear to be incapable of deaminating amino acids. The ammonia released in deamination is either transformed locally in the liver into urea, or is transported to the liver by an ammonia carrier for conversion into urea. A small part of the renal ammonia escapes into the urine, and serves to regulate its H-ion concentration.

Braunstein believes that aminopherases provide the mechanism for amino acid synthesis and deamination in tissues that contain no general deaminases, and that aminopherases also may participate in the general deamination of the Krebs and Bernheim type that occurs in kidney and in liver.

The amino group of the mono-carboxylic amino acids is transferred to keto-glutaric acid, and the glutamic acid thus formed is readily deaminated in the tissue.

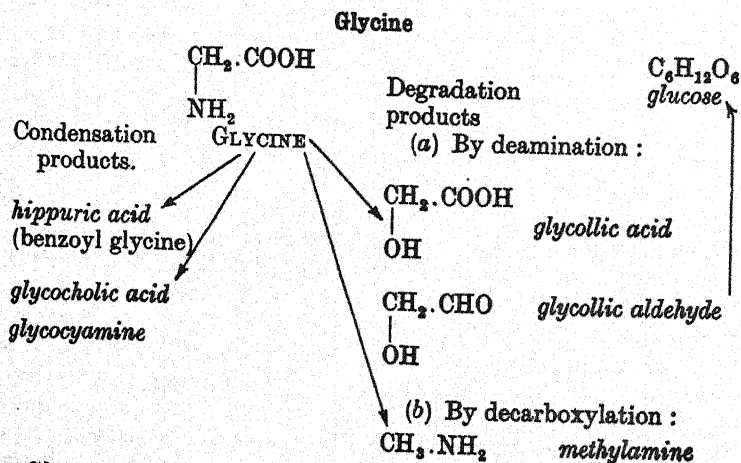
Tissue-protein Synthesis.—Since the tissue proteins are highly specific compounds, it is believed that the process of their elaboration from amino acids takes place within the cell on a catalytic surface (endo-enzyme) that in some way resembles the required protein pattern, and acts as a template or pattern-die for arrangement of the adsorbed amino acids. The protein assembled in a particular tissue may become part of the tissue structure or may be used for other purposes. Thus, the serum proteins are built up in the liver, the zymo-proteins are manufactured in the secreting glands, and caseinogen is manufactured in the mammary gland.

Summary of General Amino Acid Metabolism



Ammonia Carriers.—Although ammonia appears as an end-product of general deamination, it is too toxic and reactive a metabolite to remain in the tissues in a free form, and is taken up by appropriate carriers, such as glutamic acid, aspartic acid, adenylic acid and adenosine, or may be removed by an aminopherase system. These, or other carriers, transport ammonia to the liver for detoxication by conversion to urea.

Metabolism of the Individual Amino Acids.—Each amino acid has its own metabolic history in the organism, but few of these are known in more than outline. Inborn errors of metabolism, such as alcaptonuria, have led to the identification of intermediate products; and the increasing knowledge of the chemistry of the hormones and alkaloids has shown that many of these are amino acid derivatives.



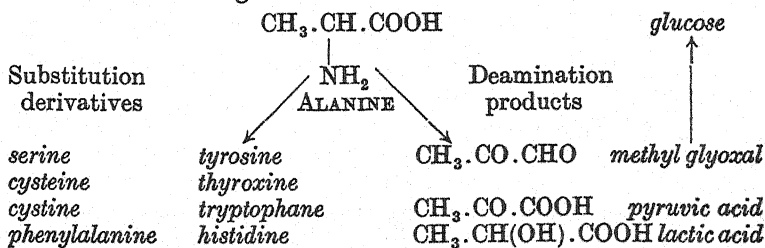
Glycine is a non-essential amino acid, the animal being able to manufacture it when required. When administered to the diabetic

subject, glycine is converted into glucose, which provides a link between protein and carbohydrate metabolism. The chief derivatives of glycine in the higher animal are hippuric acid, the detoxication solute found in urine, and glycocholic acid, found in bile.

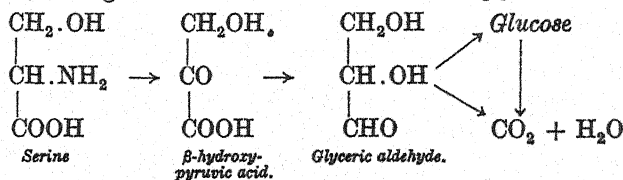
Both glycollic acid and aldehyde yield glucose when given to the diabetic animal, and are believed to be intermediate stages in the course of glycine metabolism. Methylamine is not produced in normal metabolism but may arise from alimentary putrefaction. Glycine has no effect in increasing the liver glycogen or raising the blood sugar level when fed to healthy animals. It has a marked specific metabolic effect in the dietary.

Alanine and Serine

Alanine is a precursor of glucose in the diabetic animal, and also is a non-essential acid. It is significant, however, in being related in structure, though not necessarily in biological derivation, to many more complex and important amino acids, and it readily transaminates with keto-glutaric acid.



Serine is quantitatively converted into glucose in the diabetic animal, through the intermediate formation of glyceric aldehyde.



Phenylalanine and Tyrosine

These aromatic amino acids provide the chief source of the benzene ring, which, according to Abderhalden, cannot be synthesised by higher animals. Both amino acids yield acetoacetic acid when oxidised by liver tissue or when administered to diabetic subjects, and both yield *homogentisic* acid in alcaptonuric subjects. It is concluded that phenylalanine is transformed, in part at least, into tyrosine during metabolism. In *tyrosinosis*, path A is completely

homogentisic acid both yield acetoacetic acid, and thus are possible intermediates in tyrosine metabolism. From phenylalanine or tyrosine are derived the important hormones adrenaline and thyroxine, and the vaso-pressor tyramine, and, by bacterial degradation in the intestine, cresol and phenol, which appear as sulphates in the urine.

According to Levine *et al.* (1943), the conversion of phenylalanine into tyrosine is irreversible in the human organism.

Tyrosine also can give rise to 3:4-dihydroxyphenyl alanine, an amino acid found free in plants, and by oxidative closure of the side-chain produce the melanin pigments (p. 226).

Phenylketonuria.—An excretion of phenylpyruvic acid, $C_6H_5.CH_2.CO.CO_2H$, occurs in the inherited insanity, *imbecillitas phenylpyruvica*. The output is increased by phenylalanine, but not by tyrosine administration, which implies that the two amino acids traverse different metabolic paths, or that the organism cannot dehydroxylate tyrosine. Phenylketonuria is an interesting example of a metabolic disorder associated with a mental defect.

The formation of homogentisic acid is an example of the way in which a biological reaction can proceed on lines that differ from those indicated by purely chemical considerations. In homogentisic acid, the $-OH$ at 4 in tyrosine has been replaced by H, and two new $-OH$ groups have appeared at 2 and 5, respectively. This may be the normal way in which the organism starts to unlock the benzene ring, or may merely be an unsuccessful attempt to do so in the alkaptonuric subject. Incubated with kidney or liver tissue, tyrosine is deaminated to 4-hydroxyphenyl pyruvate, which, in liver, is then broken down to acetoacetate.

Tryptophane

Tryptophane is an essential amino acid, and provides the only known source of the indole nucleus in the animal diet.

While no important biological reactant has yet been traced to tryptophane, it is probable that it supplies pyrrole nuclei for the synthesis of the porphyrins, including the essential cytochrome and hæmatin. Surplus dietary tryptophane is excreted in the urine as *kynurenine* (in rabbits), and as *kynurenic acid* (in dogs, rats, foxes and wolves).

Tryptophane is the parent of the putrefaction products *scatole* and *indole*, and their oxidation derivative *indoxyl*.

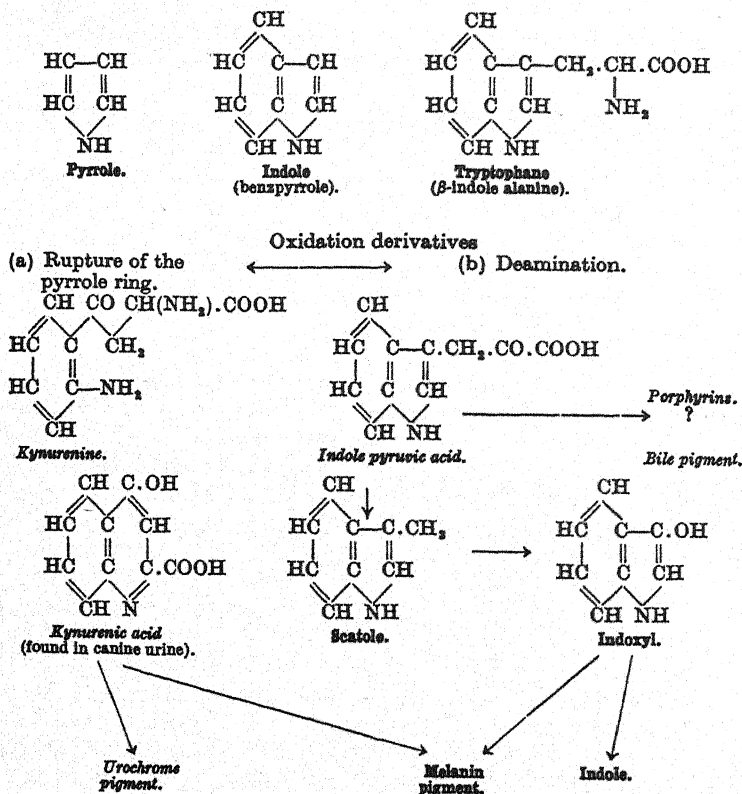
About 50 mg of indole are excreted daily, depending on the protein intake and the activity of the intestinal flora; and about 8–10 mg. are absorbed.

Indole is present in the contents of the large intestine. It gives many colour reactions, notably :

(a) With Na nitroprusside in alkaline solution, a purple colour turning blue on acidification with acetic acid.

(b) With Ehrlich's aldehyde reagent in acid solution, a red colour, stable on dilution.

(c) Boiled with xanthydrol in acetic acid, a deep violet. This reaction is not given by other biological compounds, unless a strong acid is present, when tryptophane and scatole react similarly.

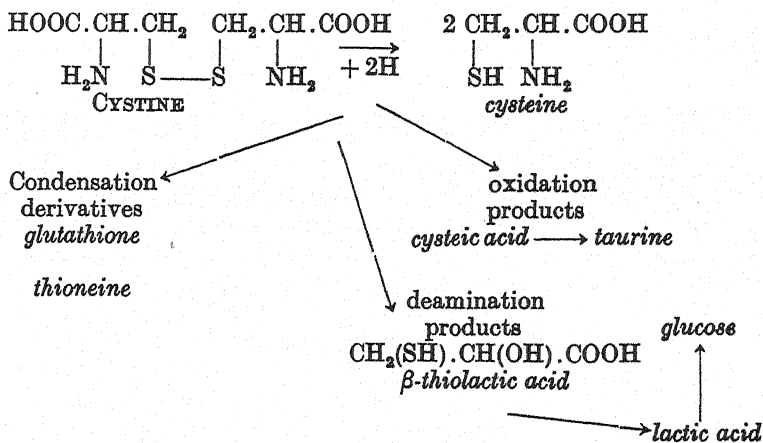


Tryptophane Metabolism

Sulphur-containing amino acids include cystine and methionine, and their derivatives, cysteine, homocysteine, homocystine, and the tripeptide glutathione.

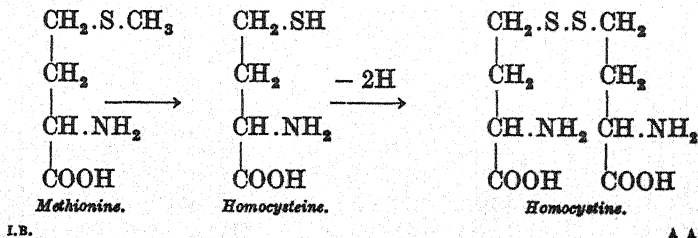
Cystine, the chief of these, is the parent of many sulphur derivatives in the organism, and is an important constituent of the dietary. On reduction it is converted into two molecules

of the amino acid cysteine, which contains sulphur as the highly reactive *thiol* group, —SH. Cysteine is completely converted into glucose when administered in phloridzin diabetes; in this respect it resembles alanine and serine. Sulphur liberated from cysteine is the chief source of the organic and inorganic sulphates.



When administered to subjects of the metabolic disease *cystinuria*, cysteine, homocysteine and methionine are excreted largely as additional cysteine; whereas administered cystine, homocysteine and glutathione are almost completely oxidised. From these observations it is concluded that cystine can be metabolised without previous reduction to cysteine, and that glutathione can be metabolised without previous hydrolysis, indicating that the metabolic history of an amino acid may depend on whether it is free or combined. Methionine, prior to its conversion into cysteine, is demethylated to form homocysteine, which may undergo condensation to homocystine or degradation to simpler products.

Methionine is the chief source of methyl groups in biological methylation.

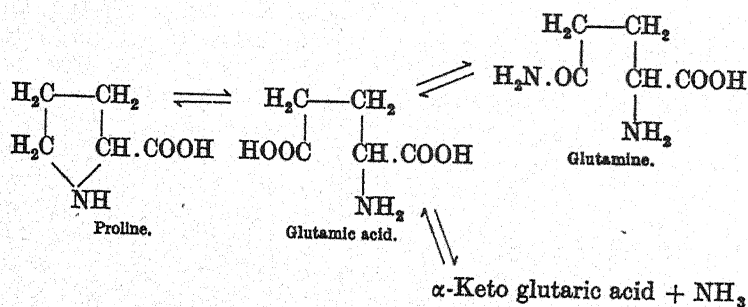


Glutamic Acid and Proline

Neither glutamic acid nor hydroxy-glutamic acid has been found essential in nutrition although the former is a component of an aminophorase system and also acts as a specific ammonia carrier in the metabolism of nervous tissue and in the formation of the urea in the liver, and is a component of the tripeptide *glutathione*.

In presence of glutamic acid, retina and brain cortex release ammonia in quantities up to 0.8 per cent. of the dry weight of the tissue, per hour, the ammonia being fixed as glutamine. A similar reaction has been observed in sheep, and rat kidney, though not in the kidney tissue of cat, dog, sheep or pigeon. Apart from this, glutamine formation appears to be restricted to the nervous system. The reaction is due to the specific enzyme, *glutaminase*, that under appropriate conditions aminates glutamic acid or deaminates glutamine. According to Hamilton (1945), glutamine is the chief amino acid in plasma (6-12 mg./100 ml.) and cardiac muscle (200 mg./100 gm.).

Proline, a non-essential glucogenic amino acid, is oxidised to glutamic acid by kidney or liver tissue, and thus forms part of the glutamic cycle. Hydroxy-proline may undergo reduction to proline, but most of it appears to follow an individual metabolic path, as shown by the fact that, unlike proline, it can be converted into acetoacetic acid by liver tissue.



The conversion of glutamic acid to α -keto glutaric acid is catalysed by a highly specific enzyme, glutamic dehydrogenase, found chiefly in liver and kidney.

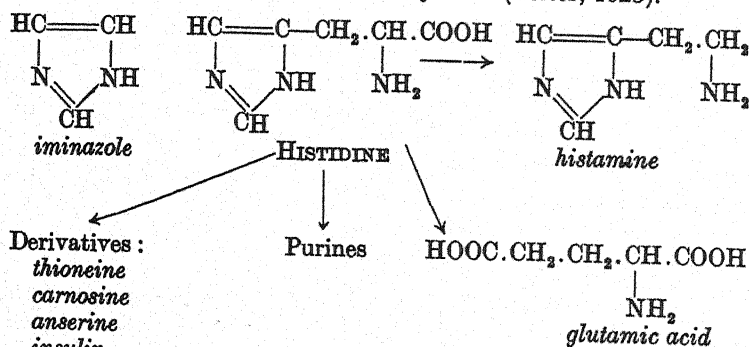
Arginine

Arginine, the amino acid derived from guanidine, is a probable constituent of all proteins, and can be synthesised by the animal body. Among mammals and many invertebrates, arginine takes

Adler to conclude that the histidinuria is due to protective inactivation of the enzyme in order to conserve the amino acid for foetal requirements.

The principal derivatives of histidine in the animal body are (i.) *thioneine*, the betaine base of thiolhistidine (p. 419), which occurs in blood corpuscles; (ii.) *carnosine*; and (iii.) *anserine*, dipeptides found in muscle (p. 430). On decarboxylation, histidine forms the corresponding amine, histamine, the capillary dilator present in tissue extracts, and the chief factor in vascular shock.

Iminazole, the cyclic component of histidine, is not utilised appreciably in animal metabolism, and can be recovered almost quantitatively from the urine after injection (Leiter, 1925).



Bromine Test for Free Histidine (Knoop, 1907; Kapeller-Adler, 1933).—Add 1 per cent. Br_2 in 33 per cent. acetic acid, drop by drop, to 2 ml. of the solution, until the yellow colour of the Br_2 just persists. After 10 minutes, add 2 ml. of 4 per cent. ammonium carbonate dissolved in concentrated aqueous ammonium hydroxide (sp. gr. 0.88) that has been diluted by half its volume of water. After another 10 minutes add about 1 gm. of sodium acetate and immerse the tube in boiling water for 5 minutes. If free histidine be present in concentrations exceeding 1 : 50,000, a purple colour develops. The test is very selective, and is not given by other iminazole derivatives, including histamine, carnosine or histidine bound in protein form.

Lysine

Lysine, or L (+)- α - ϵ -diamino caproic acid, is indispensable in animal nutrition, and is unique in the properties of its amino groups. When an intact protein is treated with an amino acid reagent, such as nitrous acid or phenyl isocyanate, subsequent hydrolysis shows that the groups preferentially attacked were the ϵ -amino groups of lysine, which, therefore, must have been free in the original protein molecule. If this be so, the terminal $-\text{NH}_3^+$ groups of lysine are

responsible for the base-neutralising power of iso-electric protein colloids, just as the ionised carboxyls, —COO^- , of the glutamic and aspartic units are responsible for the acid-binding effect.

When animals are fed with amino acids labelled by inclusion of ^{15}N , subsequent tissue analysis shows that the isotope has become distributed by trans-amination and exchange among all the other amino acid units in the proteins, with the exception of lysine, and threonine. Lysine, thus, does not exchange N with other compounds and, also, is the only natural amino acid, so far known, to be resistant to the introduction of D from body-fluids enriched with D_2O or DHO .

Weismann and Schoenheimer (1941) suggest that the stability of lysine may be due to the absence of lysine deaminase from animal tissue, or to the existence of a mechanism that condenses lysine to a 6-membered ring resistant to trans-aminations.

Threonine

In 1935, Rose observed that although hydrolysed casein could provide all the amino acids necessary for growing rats, an equivalent mixture of the nineteen amino acids, then known to occur in casein, was insufficient to maintain life. The missing factor was found to be a hitherto unrecognised amino acid, α -amino- β -hydroxy butyric acid, later named *threonine*, to show its relationship to the four-carbon sugar, D(-)-*threose*. Threonine can be oxidised by neutral periodate or lead tetra-acetate, with quantitative liberation of CH_3CHO , and thus can be estimated (Winnick, 1942). Representative percentage yields are: casein, 3.5–4.6; lactoglobulin, 5.2; gliadin, 2.8; keratin, 6.6.

Amino Acids Indispensable for Animal Maintenance.—After his discovery of tryptophane, Hopkins, in 1906, investigated its significance in animal nutrition, and later showed that young mice kept on a diet in which the only source of protein was *zein*, a maize gliadin deficient in tryptophane and lysine, failed to grow, lost weight, and died within twenty days, unless the diet was supplemented by tryptophane. Osborne and Mendel extended the work, and found that when tryptophane was added to the deficient diets, though body-weight was maintained, and life prolonged, growth was still retarded. When the diets were further supplemented by lysine, normal growth was regained. Experiments of this type showed that the higher animal was unable to synthesise certain amino acids necessary for its life.

While, no doubt, species differences exist, and general conclusions are still premature, it is believed that of the twenty-two amino acids known to occur in ordinary proteins, twelve are not essential for

The homocysteine thus formed can be reduced to homocystine, just as cysteine is reduced to cystine, or degraded to simpler products.

Trans-methylation is another example of the lability of amino acids in tissues, as also is the readiness with which they exchange with similar units in the protein molecules (Schoenheimer, 1942).

Gluco-genic Amino Acids.—Transformation of amino acids to sugars has been studied in phloridzin-treated animals, and Dakin has classified them accordingly as *gluco-genic* and *ketogenic* units (p. 154). The results, however, are not in accord with feeding experiments on normal animals. Remmert and Butts (1942) report that phenylalanine, tyrosine and histidine increase liver glycogen in rats, and decrease ketonuria due to a high fat diet, whereas arginine has little effect. The difference between these and earlier results may be due to the abnormal strain imposed by phloridzin treatment.

Indispensable Amino Acids

Name.	Significance.
1. Arginine . . .	Contains guanidine group. Component of most, if not all, proteins. Provides amido group for creatine synthesis, and yields urea by the ornithine cycle. Necessary for normal growth.
2. Histidine . . .	Source of histamine, thioneine, carnosine and other iminazole derivatives. Essential in diet for rats but not for man.
3. Phenylalanine . . .	Contains phenyl group, and is partly replaceable by tyrosine. Source of adrenaline, thyroxine and melanin pigments.
4. Tryptophane . . .	Contains indole group. Provides pyrroles necessary for hæmoglobin production.
5. Methionine . . .	Provides methyl groups and sulphur.
6. Lysine . . .	Biologically stable unit. By the terminal amino group, it confers acid-base binding property on proteins.
7. Leucine . . .	Functions unknown.
8. Iso-leucine . . .	" "
9. Threonine . . .	" "
10. Valine . . .	" "

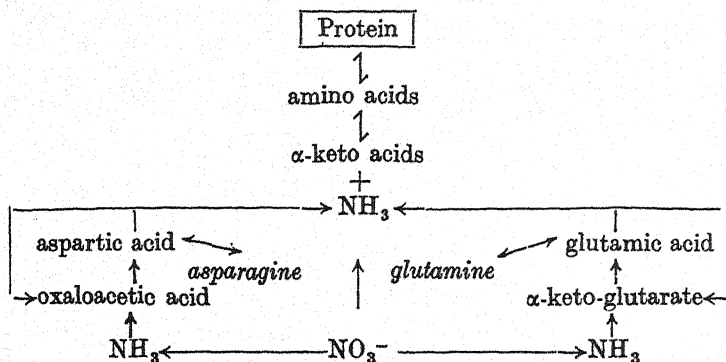
PROTEIN METABOLISM IN PLANTS

During germination, the seed proteins are broken down, liberating amino acids, which transfer their amino groups to oxaloacetic acid,

forming aspartic acid, and, subsequently, asparagine. The additional nitrogen required by the growing plant comes from the soil, chiefly as nitrate ions, which are reduced to ammonia and stored as asparagine or immediately converted to amino acids and related products.

The asparagine accumulates as a nitrogen-reserve, from which amino groups can be obtained for protein synthesis, as required.

To a lesser extent, α -keto-glutaric acid acts also as an amino acceptor, being converted to glutamic acid, and then to glutamine.



Thus, according to Chibnall (1939), the aspartic-asparagine system and the glutamic-glutamine system provide two mechanisms whereby ammonia may be fixed and stored until required, for amino acid construction. The non-nitrogenous parent acids, oxaloacetic and α -keto-glutaric, are assumed to arise during carbohydrate metabolism.

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CHAPTER 18

INTERMEDIATE METABOLISM : LIPIDES

DURING digestion, the aliphatic acids of the simple and complex lipides are liberated, and by the time the intestinal contents have reached the cæcum very little fat remains unabsorbed.

The emulsified aliphatic acids form water-soluble dispersions with the bile salts, and pass readily across the frontier of the intestinal mucosa. The glycerol is phosphorylated during its absorption in a manner comparable to the hexoses, and in the cells of the mucosa it recombines with the aliphatic acids to form fat.

Partial recombination can result in a phosphotidate, or glyceride with two aliphatic residues and one phosphoric residue, union of which with serine, choline or sphingosine, produces a phosphatide, a form in which lipides circulate in the blood.

According to Frazer (1940), unhydrolysed fat, as a fine emulsion, is absorbed directly into the lymphatics, which, after a meal, carry up to 80 per cent. of the total fat absorbed (p. 317).

The total lipide content of the blood is very variable and is made up of many components, some of which are not clearly defined. Two per cent. is maximal for normal subjects, but diabetic blood may contain as much as 20 per cent.

Lipide Distribution in Blood (Expressed in mg. per 100 ml.)

	Whole Blood.	Plasma or Serum.
Total lipides . . .	300-2,000	450-1,260
Neutral fat . . .	290- 410	190- 640
Phosphatides . . .	250- 450	175- 330
Cholesterol . . .	100- 230	100- 230

Total lipide is taken as represented by the total ether-soluble fraction of the material. Phosphatide is represented by the value for lipide phosphorus multiplied by a factor, 25.

Kirk (1938) reports average values for human plasma to be : total fat, 559 ; total phosphatide, 145 ; lecithin, 19 ; cephalin, 68 ; expressed in mg. per 100 ml.

Neutral fat travels in the blood in particles of about 1 μ in diameter, termed "chylomicrons." Like the fat in milk, they

are stabilised by being surrounded by a protective protein layer. They may be enumerated microscopically, and afford an index of the rate of fat absorption and utilisation. The fat content of the systemic blood rises in one to three hours after a meal rich in fat, reaches a peak in six or seven hours, and gradually subsides.

Structural Lipide and Storage Lipide.—In the organism the lipides are immobilised in two ways: (i.) as a structural constituent of cells and tissues, and (ii.) as the storage fat of adipose tissues. The former is determined by the growth requirements of the organism, the latter depends partly on the fat content of the diet and partly on the excess of food consumed above the nutritional requirements of the animal. The constitution of the acids in storage fat depends on the food. Oleic, palmitic, and stearic acid can be formed from excess carbohydrate, or from protein, probably through a carbohydrate intermediate stage. Unwanted lipides and sterols are excreted into the intestine, partly by the bile and partly by the mucosa of the large intestine. These make up 7–20 per cent. of the total faecal solids.

The Combustion of Fatty Acids.—Fats and their constituents provide an important source of energy to the organism. Unlike proteins, they can be stored in large amounts, and, unlike carbohydrates, their metabolism does not appear to be associated with any particular physiological process, such as muscular contraction.

The lipide content of brain, kidney, heart, spleen and lungs is almost unaffected by starvation, and must be structural in character. Starvation, however, can reduce the fat in dog muscle from 12–17 per cent. down to about 4, showing that most of it is storage lipide.

Completely oxidised, each gram of fat liberates about 9.3 kilocalories.

The liver contains about 3 per cent. of phosphatides and upwards of 1 per cent. of neutral fat.

Desaturation of Fatty Acids.—The liver is very rich in unsaturated lipides, and it is believed that fat metabolism is located chiefly in this organ. A stage in fat degradation is the formation of a glycerophosphate ester or phospholipide (p. 182), either in the intestinal mucosa during fat absorption or in the liver after absorption. These esters contain two fatty acid radicles, one of which is unsaturated in the majority of the natural phospholipides, and it is probable that desaturation of the acid radicle is a primary stage in fat degradation.

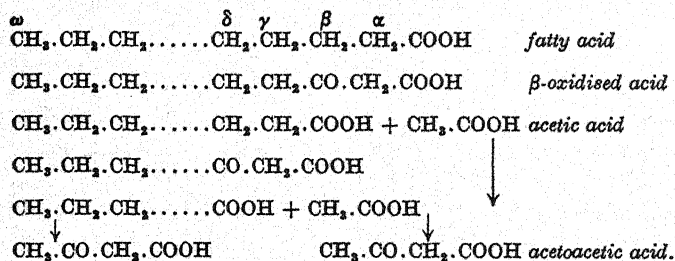
Using fats containing deuterium as an indicator, Raper and Cavanagh (1939) find a rise in the deuterium content of hepatic glycerides and phospholipides after fat absorption, and conclude that they are actively associated with early fat metabolism.

Oxidation of Fatty Acids.—The molecule of an unsaturated fatty acid such as oleic, is most likely to undergo oxidative attack at the point of unsaturation; the saturated acids, stearic and palmitic, are degraded by terminal oxidation. Fat oxidation begins chiefly, if not entirely, in the liver. The natural fatty acids almost without exception contain an even total number of carbon atoms, and to explain the process of aceto-acetic formation, Knoop proposed, in 1904, his theory of β -oxidation of the fatty acids, according to which, the point of oxidative attack is the carbon atom in the β -position, or next but one to the terminal carboxyl group. By this means the fatty acids are degraded two carbon atoms at a time, acetoacetic acid and acetic acid being the penultimate products before CO_2 and H_2O .

In 1906–08, Embden, by perfusion, showed that the liver is the chief centre for ketone production, the precursors being the natural fatty acids, some amino acids, and pyruvic acid.

In 1935, Deuel observed that the yield of ketones from fatty acids greatly exceeded the limit imposed by Knoop's theory, according to which each molecule of fatty acid is finally degraded to one molecule of acetoacetic acid. Furthermore, long-chain fatty acids yielded more ketones than short-chain acids.

Jowett and Quastel, in 1935, proposed a theory of multiple alternate oxidation, according to which the fatty chain is attacked simultaneously at several points. This was confirmed by McKay (1940), who concluded that the oxidation products are split off as acetic acid, which condenses with itself to form acetoacetic acid, the characteristic end product of hepatic fat metabolism.

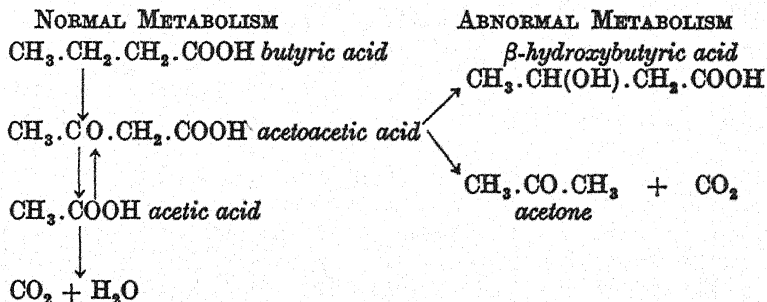


In fatty acids with an odd number of total C atoms, the terminal residue left after removal of the rest of the chain is propionic acid, $\text{CH}_3 \cdot \text{CH}_2 \cdot \text{COOH}$, which cannot form acetoacetic, and, instead, is converted into sugar.

Ketogenesis and Ketosis.—The acetoacetic acid formed in the liver, is not oxidised to any significant extent by hepatic tissue, but is sent into the circulation as a simple metabolite for utilisation

by muscle and peripheral tissues, where it competes for oxidation with the glucose provided by carbohydrate metabolism. If the glucose supply is inadequate, as explained in Chapter 16, fat metabolism is increased to meet the energy demands of the organism, and the released acetoacetic acid may exceed the metabolic capacity of the peripheral tissues. Oxidation cannot proceed rapidly, and the organism endeavours to get rid of the accumulating acetoacetic acid by two other biochemical processes. In part it is reduced to *β-hydroxybutyric acid*, and in part it is decarboxylated to *acetone*. All these metabolites enter the blood stream and appear in the urine, the conditions being termed *ketonæmia* and *ketonuria*, because two of the compounds, namely, acetoacetic acid and acetone, carry a ketone group. Normally, the ketone value of blood is less than 1 mg. per 100 ml., but in diabetic ketogenesis it may reach 300 mg. or more, corresponding to a urinary excretion of as much as 75 gm. *per diem*. Acetone is toxic, and its accumulation in the blood and tissues gives rise to the condition of *ketosis*, or ketone poisoning accompanied by acidosis, which leads to coma and death. Administration of glucose and insulin is the specific treatment of ketosis, since they provide an alternative and preferential source of energy, and thus repress the production of acetoacetic acid from fats.

Both free acetoacetic acid and acetone, which is very volatile (B.P. 56° C.), have characteristic odours. The identification of these ketones in urine is described in Chapter 23.



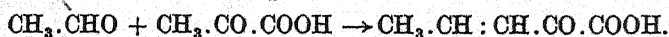
Terminal Stages in Fatty Acid Oxidation

Ketosis and ketonuria can be evoked by hypoglycæmic conditions due to general carbohydrate starvation, prolonged vomiting, or phloridzin poisoning, and may occur in untreated diabetes mellitus, and normal and toxæmic pregnancy, and after ether anæsthesia, and excessive administration of alkalis. A ketogenic factor occurs in extracts of the anterior pituitary gland, and stimulates the hepatic output of acetoacetic acid.

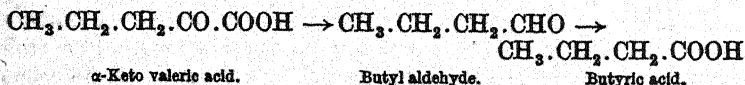
That ketosis was not uncommon in former times may be inferred from records, such as Malory's account of the death of Launcelot: "They found him stark dead, and he lay as he had smiled—and the sweetest savour about him that ever they had smelled."

Interconvertibility of Fat in the Organism.—That tissue fat can arise from dietary carbohydrate was shown experimentally by Lawes and Gilbert, in 1852, who found that pigs fed exclusively on barley acquired more body fat than could possibly have come from the fat or protein in the barley. A similar transformation is demonstrated, often unintentionally, by the human subject, and a restriction in the carbohydrate intake is a routine procedure in the treatment of obesity and over-weight conditions. Fat can also arise from protein, as shown by the work of Lusk and his colleagues on the effect of feeding excess of lean meat to dogs whose glycogen and lipide stores had been depleted by previous starvation.

Two factors are required in fat production: (i.) glycerol, and (ii.) the precursor of the fatty acids. Glycerol can arise during carbohydrate metabolism either directly from glucose or indirectly from the glucogenic amino acids. The precursor of the fatty acids is probably pyruvic acid, which is an intermediate in glucose degradation and also in alanine deamination. By decarboxylation of pyruvic acid, *acetaldehyde* is formed, and can undergo union with itself, by aldol condensation, or with pyruvic acid, to form an unsaturated 5-carbon aliphatic acid.



This may undergo reduction to the corresponding acid, α -keto valeric acid, or may be decarboxylated to an aldehyde, which then reacts with another molecule of pyruvic acid to form a 7-carbon acid. By a continuation of this process, fatty acids of increasing length can be assembled. Intermediate reactants such as these have not been found as yet in animal tissues, but the fact that milk fat contains a variety of lower members of the aliphatic series suggests that fat construction in the organism is by such stages. The preferential occurrence in nature of fatty acids containing an even total number of carbon atoms is explained by the decarboxylation and subsequent terminal oxidation of the antecedent keto acid.

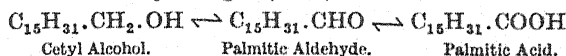


By administering fatty acids labelled by substitution of D for certain H atoms, Schoenheimer found a continuous and rapid formation and

destruction of depot acids. The rat, for example, converts *palmitic* into several other acids even when these are already provided by the diet :

Oleic acid \longleftrightarrow *stearic acid* \longleftrightarrow *palmitic acid* \longrightarrow *short-chain acids*.

It has also been shown that *cetyl alcohol* is formed from, and convertible into, *palmitic acid*, probably by way of palmitic aldehyde, which has been detected in tissues by Feulgen (1915).



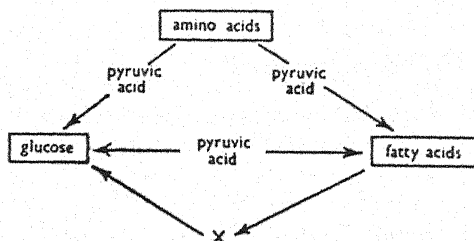
Condensation with acetaldehyde lengthens the carbon chain of palmitic aldehyde, forming β -hydroxystearic aldehyde,



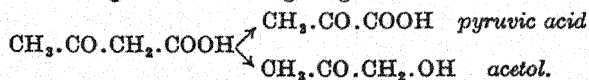
which on reduction yields stearic acid, $\text{C}_{17}\text{H}_{35}\cdot\text{COOH}$.

The possible conversion of fat to carbohydrate within the organism has been a subject of much controversy. While the glycerol component of the fat molecule can be transformed readily into liver glycogen, a carbohydrate precursor has not yet been identified clearly among the products of fatty acid metabolism. A pituitary factor in carbohydrate metabolism has been discovered, and one of its effects is ascribed to promotion of glycogenesis from fatty acids.

Inter-relationship of Protein, Carbohydrate and Fat



Weil-Malherbe (1938) reports that under anaerobic conditions rat kidney cortex forms glucose from acetoacetate, the elaboration taking place, presumably through pyruvate, and not through acetol, since compounds forming acetol are not glucogenic under similar conditions.

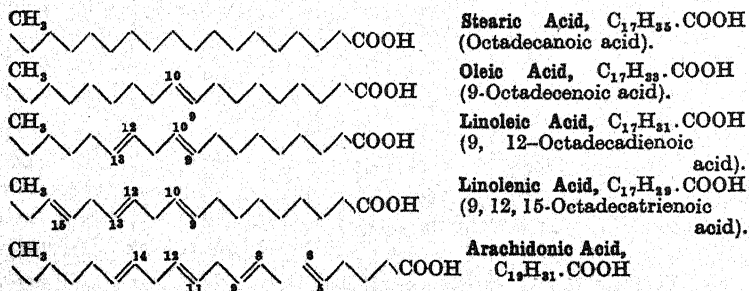


The Origin of Milk Fat.—Fat occurs in milk to the extent of 2–4 per cent. depending on the species of the animal and the dietary conditions. It differs from body fat in containing several of the lower fatty acids, about 7 per cent. of which are volatile in steam, and presumably have a special function in nutrition.

Expressed as percentage of total fatty acids, the composition of English dairy butter is: butyric acid, 4.4; caproic acid, 1.4; caprylic acid, 1.0; capric acid, 1.1; lauric acid, 3.5; myristic acid, 20.1; palmitic acid, 15.2; stearic acid, 1.1; oleic acid, 45.5 (Crowther and Hynd).

According to Graham, Jones and Kay (1936), milk fat is derived chiefly from the non-phospholipide fatty acids of the blood. Phospholipides are precursors neither of milk fats nor of milk phosphoproteins, which obtain their phosphate from the inorganic solutes of the plasma.

Indispensable Unsaturated Acids.—Burr and Burr, in 1929, found that young rats on diets free from fat, but otherwise adequate, failed to grow, and eventually developed an eczema-like scaly condition of the skin, of the body and tail. Renal lesions and hæmaturia sometimes occur. The condition can be prevented or cured by small amounts of *linoleic* or *linolenic* acid in the diet, or by the more unsaturated *arachidonic* acid (Smedley-Maclean, 1943). Although, as isotope experiments show, the organism can desaturate stearic to oleic acid, it is unable to continue the process to produce unsaturated acids that are *polyenes*, in that they have more than one unsaturated, $=CH-$, bond. The organism also is unable to assemble polyene acids from carbohydrate or other possible sources. When linoleate or arachidonate is given to the fat-starved rat, the unsaturated acid value of the liver fat increases long before any change can be found in subcutaneous or other fats, suggesting that the polyene acids are required for some special aspect of hepatic metabolism, not necessarily related to normal fat absorption or fatty acid oxidation. Arachidonic acid, first isolated, in 1909, from pig liver, by Hartley, is widely distributed, the ox adrenal being a good source. It is the most potent of the polyene acids in restoring growth to fat-starved animals, but all three acids are probably interconvertible in the organism.



The first four acids are derivatives of the 18 C linear hydrocarbon *octadecane*. Arachadonic acid, according to Dolby, *et al.* (1940), is a derivative of the 20 C hydrocarbon *eicosane*, $C_{20}H_{42}$, with four double-bonds, at 5 : 6, 8 : 9, 11 : 12 and 14 : 15, respectively.

COMPLEX LIPIDES AND LIPOIDS

Phospholipides.—Lecithin and other phospholipides of the diet are important nutritional sources of choline and of phosphoric acid, and are resolved into their components by the esterases of the small intestine previous to absorption. Within the intestinal mucosa, a re-synthesis occurs, or a new phospholipide is assembled containing units derived from the saponified fats of the diet, and as such participates in the lipide transport in the lymph and the portal blood. By use of a phosphate containing a radioactive isotope of phosphorus as indicator, Artom and his colleagues (1937) have shown that phospholipides of the lecithin and cephalin type are synthesised in large quantities during fat absorption, and accumulate in the intestinal mucosa, the liver, and to a lesser extent, the kidney, but not in the spleen, heart or skeletal muscles.

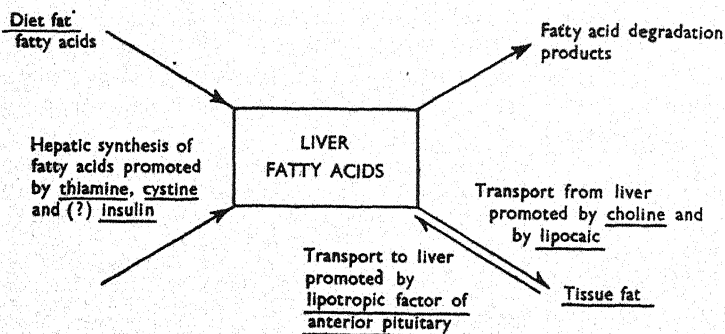
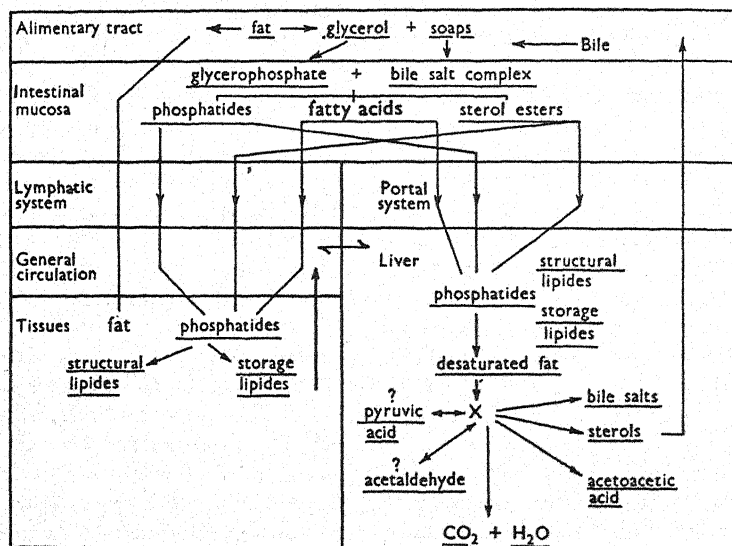
Lipotropic Factors.—Starvation causes a temporary increase in liver fat, by emergency mobilisation of fat for oxidation ; excessive and prolonged feeding with diets rich in fat may raise the fat content of the liver to 10 per cent., or more. Normally, however, the accumulation of fat in the liver is not directly determined by the fat or carbohydrate intake, but is restricted by *lipotropic*, or fat-distributing factors in the diet. These include choline, betaine, and the amino acid methionine, which is able to generate choline by transferring methyl groups to colamine, a product of serine decarboxylation. Thiamine and cystine can promote accumulation of dietary fat in the liver, probably by stimulating synthesis of fat. The lipotropic action of choline is due to its contribution to the synthesis of lecithin, the phospholipide chiefly concerned in the transport of fat.

The fatal results of diabetes in depancreatized dogs include fatty infiltration of the liver, a condition that can be prevented by feeding with raw pancreas. This lipotropic effect is not due entirely to the choline present in the pancreatic phospholipides, for Dragstead (1940) claims that it is obtained even with pancreatic extracts free from lecithin and choline, and ascribes it to the presence of a specific anti-liposis hormone, *lipocaic*, present in pancreatic tissue.

Liver fat accumulation is also stimulated by a pituitary factor that promotes transfer of depot fatty acids to the liver from the tissues (Stetten and Salcedo, 1944).

Sterols.—Cholesterol is readily and preferentially absorbed, and circulates as an ester; phytosterols are not absorbed, probably because the necessary esterifying system is absent from the intestinal mucosa. The D vitamins and the provitamin ergosterol are rapidly absorbed, but the potency of these compounds is so great that it is not easy to show how far their absorption is specific and quantitative.

Summary of Fat Metabolism



Carotinoids.—The carotenes α , β and γ , are preferentially absorbed by ruminants, but the human intestine does not discriminate

between them and the useless pigment xanthophyll, and both types of carotinoid circulate in human plasma.

Hydrocarbons.—Channon has shown that unsaponifiable hydrocarbons, phytol, higher alcohols, and liquid paraffin can be absorbed in small quantities from the intestine, depending on their solubility in bile acid mixtures. This has a practical significance on account of the widespread use of medicinal paraffins in the prolonged treatment of chronic intestinal conditions.

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CHAPTER 19

TISSUE RESPIRATION

"Life is a pure flame, and we live by an invisible sun within us."
THOMAS BROWNE.

FROM the physical standpoint, life is a peculiar and orderly way in which energy is transformed. The source of this energy is the food material of the cells, and it is released in two different ways: *degradation* and *oxidation*. By degradation is meant the fragmentation of the food molecule, usually under anaerobic conditions. By oxidation is meant the combustion of the molecule with the ultimate liberation of carbon dioxide and water. Degradation is represented by various natural fermentations, and the conversion of glycogen into lactic acid in contracting muscle. It is a primitive and uneconomical process, and provides energy for the simplest types of life.

"The development of more complicated and hence more pre-tentious forms of life became possible only after Nature discovered oxidation by molecular oxygen. This course of events is still reflected in our cells, in which we find oxidation and fermentation intimately mixed and woven into one energy-producing system."
(Szent-Györgyi, 1937.)

The term *respiration* is applied to the process whereby oxygen is utilised for the combustion of food molecules. General respiration is the sum of the activities of the entire organism as shown by the alteration in the oxygen and carbon dioxide content of the external environment; tissue or cellular respiration is the utilisation of oxygen for the combustion of cell metabolites.

The general respiration of all higher plants and animals is aerobic; lower organisms can live for varying but limited periods by incurring an oxygen debt due to the accumulation of lactate or other partially oxidised metabolites.

It was shown by Pflüger, in 1875, that frogs deprived of oxygen can survive for about seventeen hours, during the first five of which the rate of carbon dioxide elimination is normal, but subsequently decreases. These observations have been extended to other organisms, such as insects and parasitic worms, some of which can oscillate readily between aerobic and anaerobic activity according to the amount of oxygen available. The obligatory

anaerobes represent a type of monocellular organism which is unable to live in presence of free oxygen, a condition which O'Meara (1936) ascribes to the toxic effect of traces of copper in the culture media. Completely deprived of oxygen, man can only survive for about three minutes, unless specially trained to economise and endure.

Methods of Investigation.—Early work on tissue metabolism was carried out by means of extracts or suspensions of comminuted material. This has been replaced by the *tissue-slice* technique developed by Warburg and others, in which thin sections of fresh tissue are suspended in isotonic saline solutions containing various substrates. Under these conditions the structure of the tissue is preserved, and the cells remain active for several hours.

Aerobic respiration is measured directly by determining the oxygen uptake when the tissue section is placed in one cup of a Barcroft differential manometer. Anaerobic respiration is determined by incubating the material with methylene blue in a Thunberg tube containing nitrogen. This method is specially suitable for the study of dehydrogenase systems, the activity of which is found by measuring the rate of bleaching of the dye.

The Tissue Respiration Quotient.—Tissue respiration is expressed in terms of a special quotient, Q , which represents the quantity of substance produced or consumed per mg. dry weight of tissue per hour. Q , when possible, is expressed in cubic millimetres of gas (O_2 , CO_2 , NH_3) at n.t.p., and when negative indicates consumption or absorption by the tissue. Q is also qualified by the addition of suffixes, the lower of which specifies the substance transformed, and the upper denotes whether the conditions are aerobic (O_2) or anaerobic (N_2).

Thus, $Q_{O_2} = -7$ indicates that the system is consuming $7 \mu l$ (microlitres) or cu. mm. of oxygen per mg. dry weight of tissue per hour.

Similarly, $Q_{CO_2}^N = +20$ indicates that the tissue is liberating $20 \mu l$ of carbon dioxide per mg. dry weight per hour, in absence of oxygen.

A microlitre, μl , is the millionth part of a litre, or the thousandth part of a millilitre (ml.).

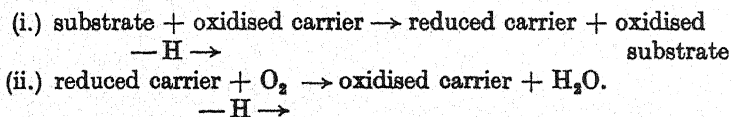
Representative Q_{O_2} values for rat tissues at body temperature are: liver, -7 ; kidney cortex, -25 ; brain cortex, -15 ; skeletal muscle, resting, -6 ; skeletal muscle, active, -40 . Holmes, from whose work these data are taken, observes that the reason for the high oxygen consumption on the part of some tissues is obviously due to the fact that they have to do physical work. "The one exception is the grey matter of the central nervous

system which, while it does no work which can be measured in terms of osmotic or mechanical energy, consumes a very considerable amount of oxygen." The human kidneys in concentrating 1.5 litres of urine in the twenty-four hours perform about 3,225 kilogram-metres of work, and have an oxygen consumption representing nearly 9 per cent of that required by the entire body when at rest.

THE RESPIRATORY PROCESS

Glucose, lactate, succinate, aliphatic and amino acids, and other substances oxidised by animal tissues are very stable in solution, and are not obviously affected by atmospheric oxygen at ordinary temperatures. In the tissues, however, they are rapidly and effectively oxidised at body temperature, which for cold-blooded animals may be as low as 10° C. These combustions are brought about by a series of chain reactions, often of surprising complexity, which include (i.) respiratory catalysts, and (ii.) respiratory carriers.

Respiratory catalysts, represented by the *dehydrogenases*, combine with the food substrates, and untabilise hydrogen atoms, which are then transferred to a series of appropriate hydrogen carriers, only the last of which interacts with free oxygen :—



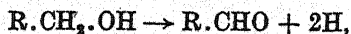
Thus, the respiratory process involves two distinct systems :—

(a) The *dehydrogenase* system (Wieland-Thunberg), which activates hydrogen in the substrate.

(b) The *carrier-oxidase* system (Warburg-Keilin), which activates molecular oxygen so that it can oxidise the activated and transferred hydrogen.

The interdependence of the two systems has been elucidated by Green and by Szent-Györgyi, who has also discovered the existence of an intermediate group of hydrogen carriers represented by the C₄ dicarboxylic acids.

Oxidation may be regarded as (i.) the removal of hydrogen, as in the conversion of alcohol to aldehyde,



or (ii.) the addition of oxygen, as in the conversion of aldehyde to acid, $\text{R.CHO} + \text{O} \rightarrow \text{R.COOH}$. In the majority of tissue oxidations, the first change undergone by the substrate is removal of hydrogen. The dehydrogenated residue, being more reactive than

the original substrate, is subsequently oxidised or hydrolysed by the action of other catalysts.

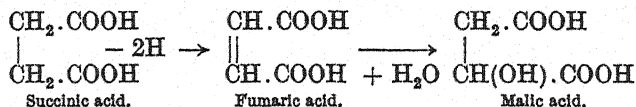
The Respiratory Catalysts

The thermo-labile respiration catalysts include :—

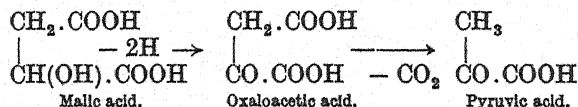
- A. *Dehydrogenases*, which activate the substrate so that hydrogen is transferred to the appropriate carrier.
- B. *Oxidases*, which activate the substrate so that it can be oxidised directly by molecular oxygen.
- C. *Peroxidases*, which transfer oxygen from peroxides to oxidisable substrates without the liberation of free oxygen.
- D. *Catalase*, which decomposes hydrogen peroxide into water and oxygen.

A. *Dehydrogenases*, dehydrases, oxido-reductases or hydrogen-transportases are widely distributed in vertebrate, invertebrate and plant tissues. Most of them are highly specific enzymes, and operate in association with respiratory carriers, which are much less specific. As a class, they are inhibited by narcotics, but not by cyanide. Important examples are :

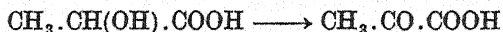
(1) **Succinic dehydrogenase**, present in most animal tissues, converts succinic acid into fumaric acid, which is subsequently hydrated to malic acid by *fumarase*, an enzyme accompanying succinic dehydrase, and one of the most powerful catalysts in the organism.



(2) **Malic dehydrogenase** oxidises malic to oxaloacetic acid, which is subsequently changed to pyruvic acid by carboxylase.

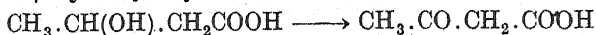


(3) **Lactic dehydrogenase**, accompanied by its co-enzyme, is present in muscle, brain and other tissues, and in yeast. It dehydrogenates lactic into pyruvic acid.

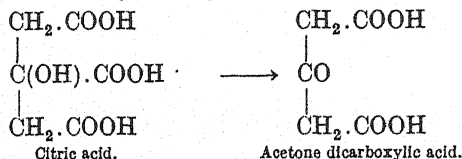


Narcotic drugs, especially barbiturates, inhibit lactic dehydrogenase in brain cortex, apparently by competing with it for the substrate (Quastel and Wheatley, 1933).

(4) **β -Hydroxybutyric dehydrogenase**, from liver and muscle, converts β -hydroxybutyric into acetoacetic acid.

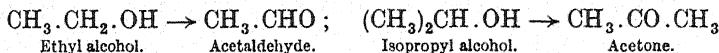


(5) **Citric dehydrogenase**, from liver, muscle and vegetable extracts, converts citric acid into acetone dicarboxylic acid, which by a second decarboxylation gives rise to acetoacetic acid.

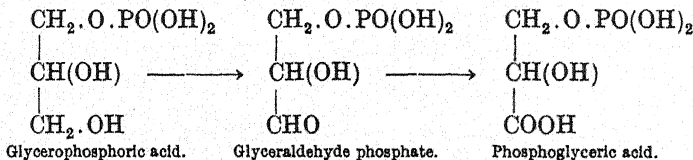


Cucumber seed is a good source of citric dehydrogenase, and, used with methylene blue as a hydrogen acceptor, forms a very delicate test for citric acid (Thunberg).

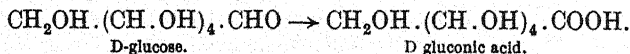
(6) **Alcohol dehydrogenase**, found in liver and kidney, dehydrogenates primary and secondary alcohols into the corresponding aldehyde or ketone. The process requires co-enzyme I.



(7) **Glycerophosphate dehydrogenase**, from yeast, converts α -glycerophosphoric acid into glyceraldehyde phosphate, and subsequently into phosphoglyceric acid, all of which participate in glycolysis. Adenosine triphosphate is the co-enzyme of the system.



(8) **Glucose dehydrogenase**, found by D. Harrison in liver, oxidises glucose into the corresponding hexonic acid.



(9) **Hexose monophosphate dehydrogenase**, from yeast and red blood cells, converts the sugar ester into the corresponding phosphohexonic acid.

(10) **Triose phosphate dehydrogenase**, in yeast and muscle, oxidises triose phosphate to phosphoglycerate, and is part of the chemical mechanism in muscle contraction and in yeast fermentation.

(11) **Hexose diphosphate dehydrogenase**, from muscle, liver, cucumber seed and other sources, activates fructose diphosphate.

These dehydrogenases activate simple substrate molecules derived from the hydrolysis or deamination of food materials. Other types of dehydrogenase activate the more complex compounds that function as hydrogen-carriers in respiration.

(12) **Co-enzyme dehydrogenases** (*diaphorases*) activate co-enzymes to effect indirect oxidation of the primary substrate by cytochrome or flavoprotein.

B. Oxidases catalyse oxidation of their substrate by free oxygen, and thus form the last member of a catalytic series. The substrate may be a food metabolite, a toxic end-product, or a hydrogen-carrier.

(1) **Cytochrome dehydrogenase**, or indophenol oxidase, accompanies cytochromes in tissues.

(2) **Monophenol oxidase**, or tyrosinase.

(3) **Polyphenol oxidase**, or laccase.

(4) **"Dopa" oxidase**.

Only the first of these oxidases is of general importance in tissue respirations. The others represent enzymes concerned in specialised aspects of plant or animal metabolism.

C. Peroxidases occur freely in tissues, rich sources being spleen, lung, liver, seedling sprouts and root vegetables, especially the horse radish. Peroxidases catalyse the transfer of hydrogen from phenolic compounds to hydrogen peroxide, without the liberation of free oxygen in the process. In the absence of a hydrogen donor, peroxidases do not decompose hydrogen peroxide.

D. Catalase is almost universal in plant and animal tissues, a particularly rich source being horse liver, from which the enzyme has been obtained in crystalline form. Catalase converts hydrogen peroxide to water and free oxygen. Hydrogen peroxide is a toxic compound, and may arise in various biological oxidations. By means of a peroxidase system it may be employed to effect subsequent dehydrogenations, or by means of catalase it may be removed rapidly.

REPRESENTATIVE OXIDATION SYSTEMS

(a) **Blood Pigments**.—(1) *Catalase effect*, liberation of molecular oxygen. Add 10 drops of 3 per cent. hydrogen peroxide to 5 ml. of blood diluted about 1:1,000. There is a rapid evolution of oxygen gas. Decomposition of the peroxide is effected by the successive reduction of the catalase iron by the peroxide, and its re-oxidation by the molecular oxygen (Keilin and Hartree, 1939), $4\text{Fe}^{+++} + 2\text{H}_2\text{O}_2 \rightarrow 4\text{Fe}^{++} + 4\text{H}^+ + 2\text{O}_2 \rightarrow 4\text{Fe}^{+++} + 2\text{H}_2\text{O} + \text{O}_2$

(2) *Peroxidase effect*, transfer of atomic oxygen to an oxygen acceptor (guaiacum). To 5 ml. of very dilute blood (1:5,000)

add 5 drops of fresh, 2 per cent. alcoholic guaiacum resin and 5 drops of the hydrogen peroxide. Mix. A blue colour develops owing to oxidation of the guaiaconic acid in the resin to "guaiacum blue." Repeat the test, having previously boiled and cooled the dilute blood. The result is still positive, showing that the catalyst is heat-stable, and not an enzyme.

A slight opalescence forms when the guaiacum reagent is added to water, and must not be mistaken for a faint positive peroxidase reaction. This can be checked by use of a control tube.

Repeat the test, using (i) benzidine, and (ii) pyramidon as oxygen acceptors instead of guaiacum, as in the methods for detecting hæmaturia (p. 459).

Pure hæmoglobin has a powerful peroxidase effect, but very little catalase effect. Conversely, hæmatin, which arises in shed blood, has a powerful catalase, but only a feeble peroxidase effect (p. 252).

Non-metallic porphyrins do not display these catalytic properties.

(b) **Milk Peroxidase.**—Dilute 1 ml. of fresh milk with 5 ml. of water. Add 5 drops of guaiacum reagent and about 10 drops of turpentine that has been "activated" by exposure to moisture and light. Shake the mixture. A blue colour develops.

Previously boiled and cooled milk no longer gives the reaction, which is due to an enzyme and not a heat-stable catalyst.

Hydrogen peroxide may be used as the substrate instead of oxidised turpentine, but is liable to destroy the milk enzyme.

Some samples of milk give the blue colour on addition of guaiacum alone, owing to the presence of traces of peroxide as well as peroxidase.

(c) **Potato Peroxidase.**—Repeat the experiments, using fresh potato scrapings, which are very rich in vegetable peroxidase, and also in catalase.

The Respiratory Carriers

Only one type of enzyme, the aerobic oxidases, is able to oxidise the substrate directly by means of free oxygen, which acts as a hydrogen acceptor and is reduced to hydrogen peroxide. The liberated peroxide is either decomposed by catalase or used for secondary reactions.

The majority of tissue oxidations are complex, and require the presence of a chain of respiratory carriers that transport hydrogen from the substrate-dehydrogenase complex to the activated oxygen. Unlike the respiratory enzymes, respiratory carriers are thermostable compounds capable of reversible oxidation-reduction. They are represented by :—

(1) *Ferroporphyrins*, or *respiratory hæmatins*; these form the prosthetic group in ferroporphyrin protein enzymes.

(2) *Flavins*, forming the prosthetic group in the flavoprotein enzymes.

(3) *Pyridine nucleotides*: cozymase (co-enzyme I, or co-dehydrogenase I), co-enzyme II (co-dehydrogenase II). These form the prosthetic group in pyridinoprotein enzymes.

(4) *Oxaloacetic acid*, in the dicarboxylic system of Szent-Györgyi.

(5) *Citric acid*, in the cycle of Krebs and Johnson.

(6) *Thiol compounds*: glutathione.

(7) *Ascorbic acid*, or vitamin C.

(8) *Thiamine*, aneurin or vitamin B₁, in co-carboxylase.

(9) *Miscellaneous respiratory pigments*, including adrenochrome, (an oxidation derivative of adrenaline) and pyocyanine.

The great majority of metabolites in animal tissues are oxidised through the intermediation of the ferroproteins and the co-enzymes.

(1) **Ferroproteins**.—From his work, begun in 1913, Warburg concluded that an organic iron compound, present as a micro-constituent of all tissues, is the primary factor in cell respiration, and operates by making free or atmospheric oxygen available for oxidising metabolites.

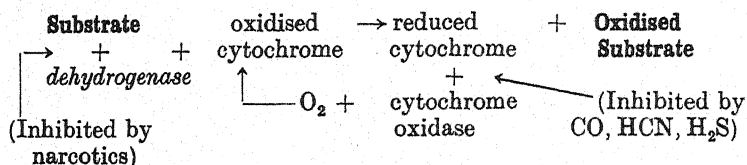
In support of this, he showed (i.) that iron occurs in concentrations of 10 γ to 100 γ per gm. of cell substance in all active tissues, and (ii.) that agents reacting with iron, such as HCN, CO and H₂S, are powerful inhibitors of tissue respiration. Cyanide in M/100 concentration inhibits about 90 per cent. of the total respiration of most tissues; the residual 10 per cent. is cyanide-stable, and due to the presence of flavoprotein carriers. Exceptional tissues are cardiac ventricle and diaphragm, with cyanide-stable respirations of 41 and 30 per cent. respectively.

Warburg concluded that his factor was a porphyrin compound, since (i.) tissues after exposure to CO display a spectrum resembling a CO-hæmatin, and (ii.) respiratory inhibition by CO is sensitive to light, being reversed by strong illumination in a manner similar to the photo-dissociation of carboxyhæmoglobin.

In 1925, Keilin showed that cytochromes occurred in all aerobic tissues, and possessed many characteristics of Warburg's factor. These cytochromes are four in number (p. 215), and are able to exist in oxidised or reduced form. Oxidised cytochrome is capable of oxidising many, but not all, tissue metabolites; and, in turn, is reoxidised by molecular oxygen in presence of the enzyme *cytochrome oxidase* (indophenol oxidase), which accompanies cytochrome.

Reoxidation of cytochrome is inhibited by HCN, CO and H₂S in

a manner similar to the inhibition of Warburg's factor, and it is concluded that the cytochromes and the Warburg factor are similar in character, and form part of the system whereby molecular oxygen becomes available for tissue respiration. According to Warburg (1934) all four compounds form a chain, the members of which are alternately oxidised to ferric or reduced to ferrous state, thus transmitting an oxidation potential to the first member of the carrier series which accepts the hydrogen from the substrate activated by dehydrogenase.



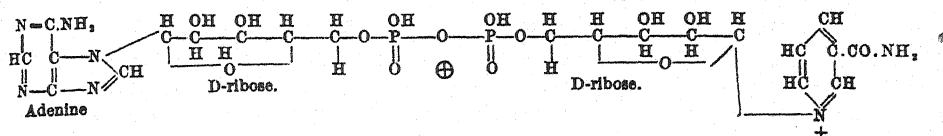
(2) **Flavoproteins.**—In 1932, Warburg and Christian isolated an enzyme from yeast, which, owing to its colour they named the "yellow enzyme" (gelbes Ferment). In presence of a co-enzyme and a dehydrogenase (Zwischenferment) the yellow enzyme catalysed the oxidation of hexosemonophosphate (Robison's ester) to phosphogluconic acid by free oxygen. The co-enzyme of the system differed from co-enzyme in having an additional phosphoric radicle. Subsequently it was shown that the cyanide-stable respiratory mechanism in yeast and other tissues included the yellow enzyme as a necessary component, and its importance in tissue respiration was recognised. The yellow enzyme is an example of a *flavoprotein*, or conjugated protein in which the prosthetic group contains a flavin.

In the yellow enzyme, the prosthetic group is the phosphate of riboflavin, or vitamin B₂ (p. 226). The flavoprotein carrier differs from hæmatin carriers in three respects: (i.) it does not contain iron and is not inhibited by CO, HCN and H₂S; (ii.) it requires the presence of an additional carrier, co-enzyme II; (iii.) it is capable of being reoxidised by free oxygen without the aid of an oxidase. At the same time, flavoprotein can work in conjunction with cytochrome to form a system containing three successive respiratory carriers.

(3) **Pyridine Nucleotides.**—These are represented by the co-enzymes I and II, also termed the co-dehydrogenases or co-dehydrases. They are compounds assembled from nicotinic amide. Their constitution may be represented diagrammatically as:—

Co-enzyme I, nicotinic amide-ribose-(phosphoric acid)₂-ribose-adenine, *di-phosphopyridine nucleotide*.

Co-enzyme II, nicotinic amide-ribose-(phosphoric acid)₃-ribose adenine, *tri-phosphopyridine nucleotide*.



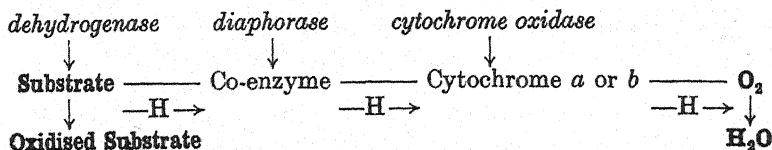
Co-dehydrogenase I (co-enzyme I, co-zymase)
Di-phosphopyridine Nucleotide

Co-dehydrogenase II has a similar formula but for the insertion of an additional —PO(OH)O— in the chain between —O— and —P— at the point \oplus , making *tri-phosphopyridine nucleotide*.

By undergoing alternate oxidation and reduction in the nicotinic nucleus, the co-enzymes act as hydrogen carriers in oxidation systems, and form a necessary link in the complicated chains of tissue activity. Oxidation of the reduced co-enzyme is catalysed by a special enzyme, *diaphorase*, a flavoprotein present in most animal tissues.

The effect of this oxidation is to transfer hydrogen from the co-enzyme to the next carrier in the series, namely, cytochrome *a* or *b*. The reduced cytochrome is then reoxidised by free oxygen, with the formation of water, the end-product of the metabolic process.

In the biological reaction, the substrate is oxidised by the transfer of hydrogen to successive carriers, the last of which reacts with oxygen.

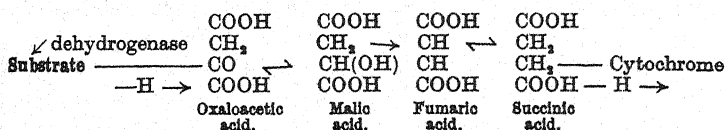


Co-enzyme I (co-zymase) is necessary in animal tissues for the activity of specific dehydrogenases that oxidise important intermediates, such as lactate, malate, triose phosphate, and ethyl alcohol.

After conjugation with the appropriate enzyme, the co-enzyme is reduced by H transferred from the attacked substrate. Nucleotidase, present in many tissues, can hydrolyse co-enzyme I, but is inhibited by the vitamin nicotinic amide.

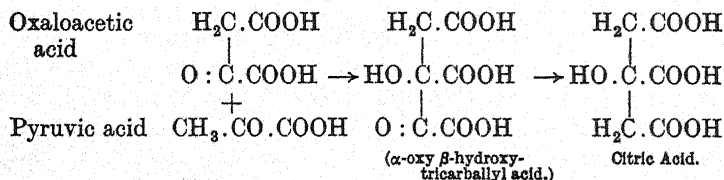
Co-enzyme II acts as a hydrogen carrier in the dehydrogenation of glutamic acid, hexosemonophosphate and glucose. All the other known systems that require a co-dehydrogenase employ co-enzyme I.

(4) **The Oxaloacetic System** (Szent-Györgyi, 1937).—The wide distribution of the three enzymes, succinic and malic dehydrogenase, and fumarase, suggests that they and their respective substrates participate in many tissue respirations. Addition of malonic acid, which inhibits succinic acid oxidation, leads to an almost complete suppression of the respiratory activity of a tissue, whereas addition of fumaric acid greatly increases respiration. From these observations, Szent-Györgyi concluded that an intermediate system, consisting of the four C_4 dicarboxylic acids, functions as a hydrogen carrier between the primary substrate and cytochrome.



In respiring muscle tissue, oxaloacetate is rapidly reduced to malate, which, in turn, can be reoxidised to oxaloacetate. This change is now known to be a necessary link in many respiration systems of animal tissue and bacteria.

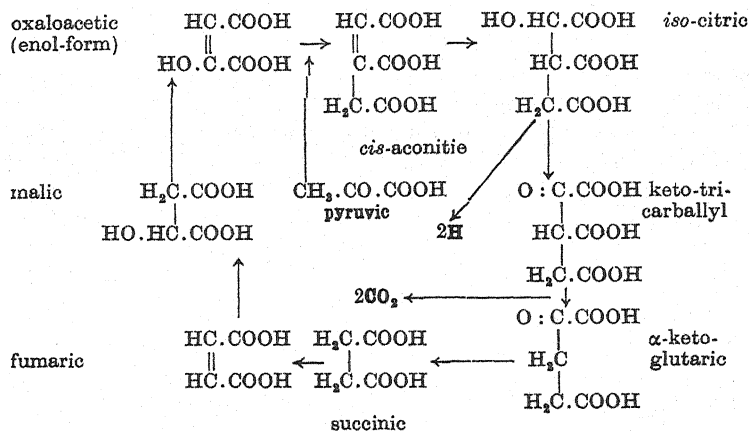
(5) **The Citric Cycle.**—Citrate is a normal constituent of body fluids and the urine, and is stored in the skeleton. Administration of pyruvate, the intermediate reactant common to sugar, amino acid, and, possibly fatty acid metabolism, results in an increased urinary output of citrate. Many tissues can form citrate by condensation of pyruvate and oxaloacetate, followed by reduction :—



In 1937, Krebs postulated the existence of a tissue respiration cycle that was fed by pyruvic acid, which reacted with oxaloacetic to yield a successive series of 6-carbon acids, until, by loss of H and CO_2 , oxaloacetic acid was regenerated ready to continue the cycle by combining with another pyruvic unit. Krebs at first assumed that citric was the first 6-carbon acid formed in the cycle, and that all carbohydrate metabolism in its final stages proceed through this citric cycle. Later work (1942) led him to conclude that *cis*-aconitic acid is the first condensation product of the oxaloacetic and pyruvic acids, and that the cycle only accounts for some 50 per cent. of carbohydrate metabolism. According to

Breusch (1943), the citric cycle is concerned principally with the degradation of β -keto acids formed by the hepatic oxidation of fatty acids, or by the reduction of acetoacetic acid.

The citric cycle involves a chain of nine reacting acids. The cycle is supplied by pyruvic acid, or some related sugar degradation product, which reacts with oxaloacetic acid to yield *cis*-aconitic acid, possibly by intermediate formation and dehydration of citric acid.



Citric Cycle

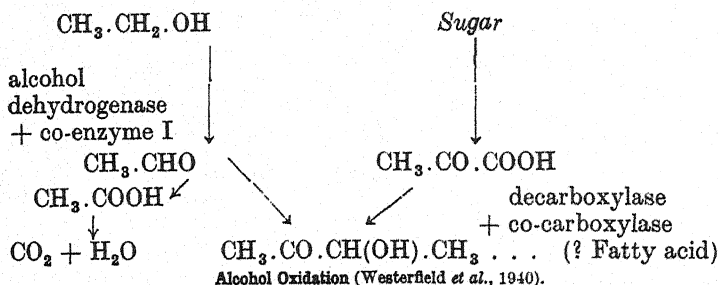
By this cycle, pyruvic acid is built up into a 6-carbon acid, which is then degraded by stages, each of which only releases a small amount of energy, until oxaloacetic acid is formed, and the cycle is ready for another revolution. The biological function of the cycle is to provide energy for coupled reactions involving synthesis, and, at the same time, to protect the tissues from injury by sudden release of energy as heat.

Citric acid occurs in most animal tissues, reaching a maximum percentage of 0.2-0.3 in fresh bone (Dickens, 1939), which is about 70 per cent. of the total body citrate. Milk contains 20-120 mg. per 100 ml.

Applications of the Citric Cycle : (1) *Carbon Dioxide Assimilation in Animal Tissues.*—The construction of urea from carbon dioxide and amino compounds in mammalian liver (Krebs and Henseleit, 1932) gave the first indication that CO_2 , a typical waste-product, is utilisable in the animal body. Since then, evidence has accumulated showing that CO_2 is assimilated during the course of various reactions, especially those concerned in the turnover of pyruvate.

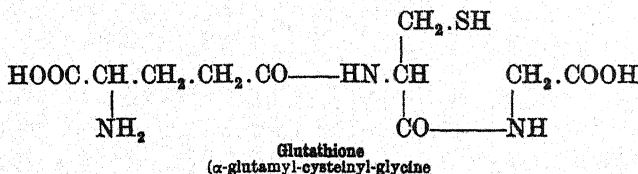
In pigeon liver, part of the citric cycle at least proceeds at a rate depending on the CO_2 pressure; this may be due to the direct carboxylation of pyruvate to oxaloacetate, a reaction demonstrated by Evans and Slotin (1940), who, using CO_2 labelled by radioactive carbon, ^{14}C , were able to locate the isotope in ketoglutarate formed from pyruvate.

(2) *Alcohol Oxidation*.—Alcohol dehydrogenase, the only enzyme known to oxidise ethyl alcohol, is located almost exclusively in the liver, and, with the aid of co-enzyme I, oxidises its substrate to acetaldehyde. Acetaldehyde is very toxic, and is kept from accumulating by being transformed into a non-aldehyde, possibly acetoin, $\text{CH}_3\text{CO}\cdot\text{CH}(\text{OH})\cdot\text{CH}_3$, or acetic acid. Alcohol metabolism is promoted by pyruvic acid, or a source of pyruvic acid, such as sugar or alanine, which suggests that a coupled reaction occurs at the acetaldehyde stage.



This scheme explains the increased oxidation of alcohol induced by vitamin B_1 (a component of co-carboxylase) or insulin, and also accounts for the increased demand for nicotinic amide (a component of co-enzyme I), and the high incidence of polyneuritis and pellagra-like conditions in chronic alcoholism.

(6) **Glutathione** is a natural tripeptide derived from glutamic acid, cysteine, and glycine. It is very widely distributed in animal tissues and forms part of the oxidation-reduction equipment.



Sources.—Glutathione was isolated in 1921 from yeast, muscle, and mammalian liver by Hopkins, and identified as the compound responsible for Mörner's cysteine reaction—the development of a violet colour when the tissue is treated with sodium nitroprusside

(nitrosoferricyanide) and ammonium hydroxide. The reaction is due to the *thiol* or *sulphydryl group*, $-\text{SH}$, and is given by most animal tissues. Glutathione is almost universally distributed in the animal kingdom, and also occurs in yeasts, fungi, and bacteria. It is present in blood corpuscles, but absent from blood serum.

Estimated glutathione percentages of fresh tissues are : yeast, 0.15-0.2 ; rabbit liver, 0.18-0.35 ; skeletal muscle, 0.045 ; kidney, 0.15 ; blood plasma, 0.0 ; whole blood, 0.04.

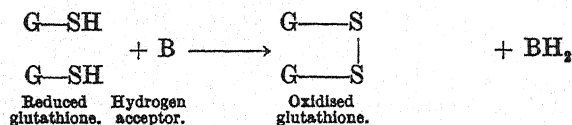
Glutathione may be obtained by the rapid extraction of pressed baker's yeast with 0.1 per cent. acetic acid, and subsequent precipitation with neutral lead acetate followed by mercuric sulphate. After removal of the metals by H_2S , the peptide is selectively precipitated by addition of cuprous oxide (Hopkins, 1929). The glutathione content of tissues may be estimated by titrating with 0.001 N iodine the solution that was obtained by exhaustive extraction of the material with 10 per cent. trichloroacetic acid until the extract no longer gave a violet colour with nitroprusside and ammonia. The method is not specific, any other labile thiol compound reacts in a similar fashion.

It is concluded, however, that glutathione is the chief thiol compound in tissues ; cysteine, which is next in importance, is probably less than one-twentieth the value of glutathione. Glutathione is a colourless, crystalline solid. It is very soluble in water and in dilute alcohol. These solutions are unstable, and if slightly alkaline, readily oxidise to form the double sulphide :—



The change is analogous to the conversion of cysteine into cystine. This auto-oxidation of glutathione is attributed by Voegtlin (1931) to the presence of minute traces of copper.

Significance of Glutathione.—The outstanding biochemical property of glutathione is its ability to assume either of two forms under conditions that obtain in living tissues :—



Animal tissues contain systems that vigorously oxidise the reduced glutathione, and systems that vigorously reduce the oxidised glutathione by means of molecular oxygen ; hence the tripeptide is capable of acting as an oxygen carrier.

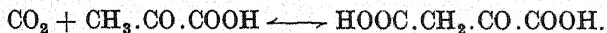
Tissue glutathione is chiefly in the reduced form, as shown by the positive nitroprusside test. The reducing agent is unknown, and

cannot be traced to one of the familiar dehydrase systems (succinoxidase, xanthine-oxidase, lactic-oxidase, citric-oxidase), and the high thermostability of the agent indicates that it is not an enzyme. Reduced glutathione in neutral or slightly alkaline solution is auto-oxidisable, depending on the presence of copper or iron. Since both these metals are invariably present in living cells as micro-constituents it is possible that their exposure or concealment may be a means whereby the oxygen uptake by glutathione is determined. G—SH in acid solutions of about pH 4 is not auto-oxidisable, even in presence of added copper or iron. Yet on the addition of an acid emulsion of unsaturated fats or fatty acids there is a steady and continuous uptake of oxygen that is far in excess of the amount that would be required to convert all the G—SH into G—S—S—G, showing that the tripeptide must have catalysed the aerobic oxidation of the lipide.

The almost universal distribution of glutathione shows it must be of great importance. For example, it is necessary for the hydration of methyl glyoxal to lactic acid by the enzyme glyoxalase, and thus may form part of a mechanism of carbohydrate fermentation.

(7) **Ascorbic acid**, vitamin C, can act as an oxygen carrier for oxidation of glutathione (Hopkins and Morgan, 1936). Ascorbic acid is oxidised by the enzyme *ascorbic oxidase*, obtained from plant tissue, where the acid presumably forms part of a respiration system. Oxidised ascorbic acid is rapidly reduced by glutathione, and thus is protected against oxidation by free oxygen or by ascorbic oxidase. Ascorbic acid is such a powerful reducing agent that it must be regarded as a potential oxygen acceptor in many forms of metabolism.

(8) **Thiamine**, aneurin or vitamin B₁ is directly concerned with intermediate carbohydrate metabolism, and, in the form of its diphosphate, *co-carboxylase*, it is necessary for the carboxylation of pyruvate to oxaloacetate in liver and other tissues :—



Co-carboxylase is also necessary for the decarboxylation of pyruvate by yeast, and, presumably, for the converse assimilation of CO₂ by plants. Hence, the same vitamin provides the agent for the oxidative removal of pyruvate, by the citric cycle in animal tissue, and the non-oxidative destruction of pyruvate, by yeast. In the organism, vitamin B₁ circulates in free form or as the monophosphate, and is converted to the co-enzyme locally within the tissues. The co-carboxylase in the blood is restricted entirely to the blood cells.

Oxidation Inhibitors

Two classes of compounds inhibit cell respiration : (1) cyanide, hydrogen sulphide, sodium azide, and carbon monoxide in very low concentrations act chiefly by preventing cytochrome oxidation by cytochrome oxidase ; (2) narcotics, such as urethane, act in high concentrations by inhibiting dehydrogenase systems that activate the primary substrates. Oxidation-inhibition and narcosis are distinct but not necessarily unrelated phenomena, since narcosis can be induced in anaerobic cells, and narcotics do not inhibit the oxidation of various metabolites by brain cortex, with the important exceptions of glucose, lactate and pyruvate. According to Quastel, narcotics are adsorbed from the cerebro-spinal fluid at specific areas in the nervous system, and by inhibiting local oxidation of glucose, lactate or pyruvate, lessen the supply of energy available for the functional activity of the cells.

Classification of Oxidases and Dehydrogenases

I. Oxidases.

- (1) Presumably activate oxygen.
- (2) Do not reduce dyes.
- (3) Do not act in absence of oxygen.
- (4) Catalyse direct reaction of metabolites with oxygen
- (5) Produce H_2O_2 .
- (6) Inhibited by cyanide.
- (7) Require neither co-enzyme nor cytochrome.

Examples : (a) Cytochrome oxidase (indophenol oxidase).

(b) Tyrosinase (monophenol oxidase).

(c) "Dopa" oxidase (dihydroxyphenylalanine oxidase).

(d) Polyphenol oxidase.

(e) Ascorbic acid oxidase.

II. Peroxidases.

- (1) Oxidise substrates by means of H_2O_2 .
- (2) Inhibited by cyanide.

III. Catalase.

- (1) Decomposes H_2O_2 to O_2 and H_2O .
- (2) Inhibited by cyanide.
- (3) May have peroxidase activity.

IV. Aerobic Dehydrogenases.

- (1) Activate hydrogen of metabolites.
- (2) Reduce dyes.

- (3) Act in absence of oxygen when suitable dyes are present.
- (4) Catalyse direct reaction between metabolites and oxygen.
- (5) Produce peroxide in presence of oxygen.
- (6) May or may not be inhibited by cyanide.
- (7) Require neither co-enzyme nor cytochrome.

Examples : (a) Xanthine oxidase (Schardinger enzyme).
 (b) Tyramine dehydrogenase.
 (c) Uricase.

V. Anaerobic Dehydrogenases (classified according to the first carrier).

- (1) Activate hydrogen of the metabolites.
 - (2) Catalyse reaction between metabolites and carriers.
- A. Cytochrome-linked Dehydrogenases :
- (a) Succinic dehydrogenase.
 - (b) α -glycerophosphate dehydrogenase.
- B. Co-enzyme I-linked Dehydrogenases :
- (a) Lactic dehydrogenase.
 - (b) Malic dehydrogenase.
 - (c) β -hydroxybutyric dehydrogenase.
 - (d) Citric dehydrogenase.
 - (e) Glucose dehydrogenase.
 - (f) Alcohol dehydrogenase.
 - (g) Aldehyde mutase.
 - (h) Triose phosphate dehydrogenase.
 - (i) Dihydroxyacetone dehydrogenase.
 - (j) L(+)-glutamic acid dehydrogenase.
- C. Co-enzyme II-linked Dehydrogenases :
- (a) Glucose dehydrogenase.
 - (b) Hexose monophosphate dehydrogenase.
- D. Unclassified :
- (a) Choline dehydrogenase.
 - (b) α -keto acid dehydrogenases.
 - (c) Fatty acid dehydrogenases.
 - (d) Histaminase.

Summary of the Dehydrogenase—Carrier Systems

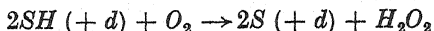
(Classification according to D. E. Green)

SH = primary substrate for oxidation ; *S* = oxidised substrate.
d = primary dehydrogenase ; *cy.d* = cytochrome dehydrogenase (or oxidase).
CyH = reduced cytochrome ; *Cy* = oxidised cytochrome.

CdH = reduced co-dehydrogenase (co-enzyme); Cd = oxidised co-dehydrogenase.

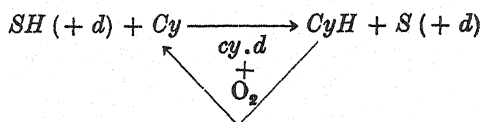
FH = reduced flavoprotein; F = oxidised flavoprotein.

(i.) *Aerobic dehydrogenases*: react directly with molecular oxygen, produce H_2O_2 , and do not require a co-enzyme.



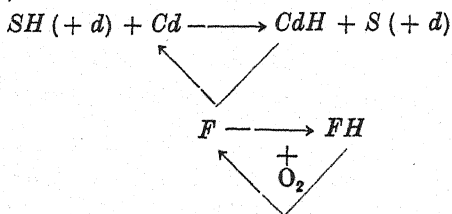
(ii.) *Simple cytochrome systems*: react with molecular oxygen through the intermediary of cytochrome activated by cytochrome oxidase. Require neither flavoprotein nor co-enzyme.

E.g.—*Dehydrogenases* of α -glycerophosphate, succinate, and (for the yeast enzyme) lactate.

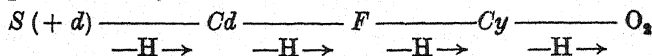


(iii.) *Simple co-dehydrogenase systems*: react with molecular oxygen through the successive intermediaries of co-dehydrogenase I or II and flavoprotein. Do not require cytochrome.

E.g.—(a) Co-dehydrogenase I systems: *dehydrogenases* of malate, fumarate, glucose (liver), and lactate (muscle). (b) Co-dehydrogenase II systems: *dehydrogenase* of hexose phosphate (yeast and red blood cells).



(iv.) *Cytochrome-flavoprotein systems*: react with molecular oxygen through the successive intermediaries of co-dehydrogenase, flavoprotein and cytochrome.



OXIDATION-REDUCTION POTENTIAL

The majority of biochemical decompositions are oxidative in nature, and the tendency for a reactant to undergo oxidation or reduction can be measured in terms of a potential. Every oxidation

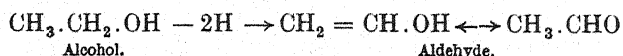
involves a corresponding reduction of some other reactant, and may be regarded as an interaction between an *oxygen donator* and an *oxygen acceptor*, owing to difference in oxidation-reduction potential.

These potentials may be determined electrometrically by a method analogous to that adopted in measuring H-ion potentials, but there are difficulties in obtaining significant results.

For example, many oxidations are highly sensitive to changes in pH, and are enormously affected by the presence of traces of catalytic metals, notably iron and copper. Also, the method is based on the assumption that the reaction is reversible. Now, biological metabolism is typically irreversible. It is not possible by feeding an animal with carbon dioxide, water, and urea to persuade it to reconvert these end-products into protein.

Hence, instead of trying to determine the oxidation-reduction, or *redox* potentials of living tissues, investigators have sought to isolate simple chemical systems capable of undergoing reversible oxidative changes, and to measure their potential under conditions similar to those occurring in the parent tissue.

The Electrochemical Theory of Oxidation-Reduction.—Formerly the phenomena of oxidation and reduction were regarded as direct additions of oxygen or hydrogen, though conversion of alcohol into aldehyde was best explained by the assumed direct removal of hydrogen :—



Elements with variable valency, such as iron and copper, however, can be oxidised or reduced without the acquisition or loss of oxygen ; and the concept was extended to include oxidative *increase* or reductive *decrease in valency*. The valency of an element is measured by its ability to gain or lose electrons. When an atom of ferrous iron, Fe⁺⁺, passes into the ferric state, Fe⁺⁺⁺, there is an increase in the positive charge due to loss of the negatively-charged electron ; and when ferric iron becomes ferrous iron there is a gain of one electron, and a consequent decrease in the positive charge. That is to say, the oxidation of an element involves the loss of one or more electrons, and the reduction of an element involves the gain of one or more electrons.

When hydrogen is oxidised to form water, each hydrogen atom loses an electron, and each oxygen atom is reduced by the gain of two electrons. The transfer of electrons in oxidation-reduction reactions is from the reducing agent to the oxidising agent.

A *reducing agent* is a substance capable of donating electrons.

An *oxidising agent* is a substance capable of accepting electrons. When a reducing agent and an oxidising agent are mixed in one solution, electron exchange takes place directly between the two reactants. If, however, the reactants are in separate containers connected by an inverted U-tube with an electrolyte, such as KCl, nothing happens until an external circuit is established by means of a conductor with an electrode immersed in each container.

Under these conditions, the electrons from the reducing agent are taken up by its electrode and travel along the conductor to the electrode immersed in the oxidising agent, and the consequence is the same as if the reactants had been mixed in the same container. This arrangement constitutes an electrolytic unit for the production of a current by a chemical reaction, and is exemplified by the various types of primary cells and "dry batteries" in common use.

Electrode Potential.—The potential difference set up between the two solutions in the oxidation-reduction unit is determined by the electron-donating tendency or "electron-fugacity" on the part of the reducing agent. This can be measured electrometrically by means of an inert electrode immersed in a solution of the reducing agent, the other solution being replaced by a hydrogen-electrode cell containing a buffer solution at the same pH as the reducing agent. Under these conditions, any potential difference between the two electrodes will be due to differences in electron concentration and not H-ion concentration. The potential difference, when referred to the standard hydrogen electrode and measured in volts is termed the *reduction potential*, E , or E_H .

The value of E depends both on the concentration of the reducing agent and the extent to which it has undergone oxidation owing to loss of electrons; consequently, the zero value, E_0 , is obtained when the reducing agent has come into equilibrium with its oxidised form, and both are present in equal concentration. Adopting the logarithmic notation similar to that used in expressing H-ion concentrations,

$$E = E_0 + k \log \frac{[\text{Oxidised form}]}{[\text{Reduced form}]}$$

where k is a constant depending on the absolute temperature. For a quinhydrone system at a uniform pH and at 30° C., the value of k is 0.03. Just as pH is determined by a balance between H-ions and OH-ions, E is determined by a balance between the oxidised and reduced forms of the reactant in the solution. The values of E_0 for a number of oxidation-reduction systems have been estimated, and a scale of voltage intensities can be prepared, the higher representatives of which will oxidise the lower representatives.

Oxidation-Reduction Potentials

System.	Solvent.	Temperature.	E_o .
Mn \cdots /Mn \cdots	15N H $_2$ SO $_4$	12°	+ 1.511
Fe \cdots /Fe \cdots	0.1N HCl	25°	+ 0.743
Quinhydrone/hydroquinone	0.1N HCl	18°	+ 0.618
I $_2$ /I $^-$	H $_2$ O	—	+ 0.54
Cu \cdots /Cu \cdots	H $_2$ O	—	+ 0.18
V \cdots /V \cdots	N H $_2$ SO $_4$	18°	— 0.204
Sn \cdots /Sn \cdots	N HCl	25°	— 0.426
Sn \cdots /Sn \cdots	0.6N NaOH	18°	— 0.854

Whether a substance is an oxidising or a reducing agent is determined by its ability to accept or donate electrons. Systems with a high positive value of E_o are regarded as oxidising agents since they can accept electrons readily; and systems with a high negative value of E_o are generally described as reducing agents.

Oxidation-Reduction Indicators.—*Redox Indicators.*—Many dye-stuffs and natural pigments are converted to colourless *leuco* compounds on reduction. The change is reversible, and the colour intensity of the compound is determined by proportions of the components present.

Methylene blue is a familiar example of a pigment readily bleached by reducing agents. Its sensitivity varies greatly with the hydrogen ion concentration, as shown by the value of its E_o at 30°, which ranges from + 0.1 (pH 5.0) to — 0.5 (pH 9.0). Under constant conditions of temperature and pH the degree of reduction can be found from the colour intensity of the mixture.

Colour Intensity of Methylene Blue at pH 7.0 and 30° C.

E_o	— 0.01	— 0.003	+ 0.011.	+ 0.025.	+ 0.062 volta.
Colour	none	intermediate shade			deep blue
Oxidised form	0.1%	25%	50%	75%	98%
Leuco form	99.9%	75%	50%	25%	2%

A series of oxidation-reduction indicators of varying degrees of sensitivity has been prepared, and may be applied to the detection and estimation of biological reactants.

Important examples are : (i.) The use of methylene blue as a hydrogen acceptor in dehydrogenase systems. (ii.) The *nadi* reagent, which led to the elucidation of the Warburg-Keilin system. (iii.) The application of methylene blue as an internal indicator in sugar estimation by Lane and Eynon. (iv.) The estimation of ascorbic acid by titration with dichlor-indophenol.

Summary.—As Szent-Györgyi has pointed out (*Harvey Lectures*, 1938–39), the primary fuel of life is hydrogen. All organic food is essentially but a fixed form of hydrogen, and all the energy that supports life is derived from the oxidation of hydrogen to water. This is accomplished by the cytochrome system of Keilin and the Warburg catalyst. One at least of the cytochromes contains iron. This iron is alternately oxidised and reduced. Ferrous iron contains one electron more than ferric iron, and the transference of this electron activates the series of interlinked reactions that constitute the respiratory process.

ENERGY EXCHANGE IN BIOLOGICAL REACTIONS

Every chemical molecule carries potential energy in the bonds uniting the constituent atoms. When a reaction takes place, molecules are broken down, and energy is transferred. Two types of change can occur : (1) *exergonic*, or exothermic reactions, in which the products have a lower potential energy value than the original reactants, the surplus energy having escaped from the system, usually in the form of heat ; (2) *endergonic*, or endothermic reactions, in which energy has been taken in from an outside source, and the products have a higher potential energy value than the reactants. In general, degradations, hydrolyses, and combustions, are exergonic, and proceed spontaneously ; condensations and syntheses are endergonic, and must be driven. The operations of life are characterised by the unique way in which exergonic reactions are used to provide energy for coupled endergonic reactions, such as the construction of protein. The exergonic process is a complex system of interlinked cycles, each of which only releases a small amount of energy, so as not to overload or burn up the cell structures. Animals, fungi, and other heterotrophes, derive their energy entirely from exergonic oxidation of nutrients. Green plants and a few pigmented organisms, have, in addition, a photosynthetic mechanism that enables them to capture solar energy, and use it to drive endergonic reactions. The process is not highly efficient, and owes its importance to its universality throughout the biosphere.

Of the total energy from the sun that reaches the world, 35 per cent. is reflected, mostly from clouds, and 65 per cent. is absorbed by the

earth's surface, only 0.07 per cent. being trapped by plants. According to Ashby (1933), the heat-energy radiations falling on an acre field in nine months represent a calorie-value equal to 1,476 tons of coal. If that acre be covered with wheat, the calorie equivalent of 0.623 tons of coal is fixed as starch and other oxidisable organic compounds, the efficiency of wheat as a transformer of solar energy being about 0.04 per cent.

Energy Units.—All other forms of energy can be transformed quantitatively into, and measured as heat. The international heat unit is the *gram-calorie*, and is defined as the quantity of heat required to raise the temperature of 1 gm. of pure water from 14.5° C. to 15.5° C. In nutritional work, a larger unit is used namely the *kilocalorie*, or *kilogram-calorie*, which is equivalent to 1,000 gm. calories. Confusion may arise from the inconsistent use of "Calorie," with a large "C," to distinguish the kilocalorie, or "large calorie," from the gm. calorie, or "small calorie," and the term always should be qualified.

Translated into other energy units, 1 gm. calorie = 4.182×10^7 ergs.

Energy of Combustion and Formation.—Organic food materials, other than proteins, are completely oxidised in the animal, and their energy value can be found by measuring the heat liberated when the material is burned to CO_2 and H_2O , under specified conditions. In protein metabolism, the amino groups are only degraded as far as urea, a less troublesome end-product than HNO_3 , hence the complete combustion values found for proteins must be corrected by subtracting the combustion value for their urea equivalent.

Combustion is carried out by electric ignition of a known weight of dry material in oxygen in a closed constant-volume calorimeter of the Berthelot "bomb" pattern. By means of an open, constant-pressure calorimeter of the Benedict-Fox type (1925), it is possible to measure the volume of O_2 required, and the volumes of CO_2 and H_2O produced by the combustion. Knowing the heat of combustion of a compound, and the heats of combustion of its constituent elements, it is possible to calculate the *energy of formation* of the compound.

When a gram-molecule (180 gm.) of dry fructose is completely oxidised it yields CO_2 , H_2O and 675 kilocal. When its equivalent 72 gm. of carbon and 12 gm. of hydrogen are oxidised, they yield the same amounts of CO_2 and H_2O , together with 975 kilocal.; the difference, 200 kilocal., is taken as the energy of formation of a gm. molecule of fructose from its elements. Thus, knowing the molecular heats of combustion of intermediate reactants, it is possible to calculate the energy of formation of, say, glycogen from lactic acid, and urea from CO_2 and NH_3 .

*Representative Heats of Combustion**Products from 1 gm. of dry material*

	Kilocals.	CO ₂ , ml.	O ₂ used, ml.	CO ₂ /O ₂ = R.Q.	Heat evolved, kilocal. per litre. CO ₂ O ₂	
Starch . . .	4.23	823.9	823.9	1.00	5.06	5.06
Sucrose . . .	3.96	785.5	785.5	1.00	5.04	5.04
Glucose . . .	3.79	746.2	746.2	1.00	5.01	5.01
Lactic acid . . .	3.62	745.9	745.9	1.00	4.85	4.85
Fat, average . . .	9.5	1,431	2,013	0.71	4.72	6.64
Ethyl alcohol . . .	7.08	972.9	1,459	0.67	4.85	7.28
Protein, as far as urea . . .	4.4	773.8	956.9	0.81	4.60	5.69

Representative values, in kilocal. per gm., for materials of industrial interest are : hydrogen, 34; paraffin oil, 9.8; butter, 9.2; carbon, 7.8; coal, 7; sulphur, 2.3; iron, 1.57.

The respiratory quotient, R.Q., is the ratio of the volume of CO₂ produced to that of O₂ used in combustion. The value depends on the proportion of oxygen in the compound, and ranges from 1.00 (carbohydrates) down to 0.7 (long-chain fatty acids).

Animal Calorimetry.—Calorimeters capable of measuring the heat given off by a living animal were devised, in 1780, by the French physicists Lavoisier and Laplace, who thereby showed that animal heat is derived from the combustion of food material. More than a century later, Rubner, using a very accurate apparatus, demonstrated that, within an error of 1 per cent., “energy is neither created nor destroyed in the animal body, but merely transformed.” This, the *first law of animal thermodynamics*, has been confirmed by many subsequent investigators. Calorimeters capable of accommodating the human subject were constructed in America, in 1899, by Atwater and Rosa, and have been modified by F. G. Benedict and his colleagues, and by DuBois.

Descriptions of the complete apparatus will be found in standard works on physiology and on nutrition. It is very elaborate, and comprises an adiabatic, or heat-insulated, respiration room, kept at human-body temperature by means of water-jackets. The composition of the atmosphere is kept uniform by admission of O₂, and removal of CO₂ and H₂O vapour. The rate of animal heat production is found by measuring the volume of the outflow water and the small temperature difference between the outflow and inflow, when the water circulates at a speed sufficient to compensate for the heat produced by the subject.

By this means, the energy output of the subject during sleep, and various forms of measurable activity, is recorded and equated with the net potential energy intake, and change in body-weight during the period of the experiment. For short duration experiments, in which the subject's weight does not change appreciably, the net energy intake is the difference between the calorie value of the food consumed and the oxidisable waste material excreted by the kidneys and intestine.

Indirect Calorimetry.—For clinical or outdoor work, direct calorimetry is impracticable, and energy-turnover is found indirectly by measuring the volume of O_2 consumed or CO_2 produced during a given period.

In open-circuit methods, the subject wears a mask with a valve that allows O_2 to enter from the atmosphere, but diverts the expired air to a bag or gasometer, where it is collected, measured and analysed at the end of the experiment. In closed-circuit indirect methods, such as those of Benedict and Roth, O_2 is supplied from a collapsible self-recording container, and the expired CO_2 and H_2O are absorbed by soda-lime. By a pointer, attached to the O_2 container and writing on a revolving drum, the instrument can be made self-recording during the short, 10–20 minute duration of the test.

The *respiration rate*, or rate of O_2 consumption or CO_2 production, indicates the quantity of material metabolised in a given period. The *respiratory quotient*, CO_2/O_2 , as found by comparative analysis of inspired and expired air for O_2 used and CO_2 produced, indicates the quality, or type of the material oxidised. Both the rate and the quotient must be known for exact work, but simplifying assumptions can be made.

In short-duration closed-circuit calorimetry of resting subjects, it is assumed that the respiratory quotient is 0.82, which is the average value under these conditions, and corresponds to a release of 4.81 kilocal. for each litre of O_2 consumed. Metabolism, however, is never entirely restricted to one type of compound, but involves intermediate reactants, such as pyruvate, that are common to sugar, fat and protein breakdown. For accurate work and long-duration experiments, it is necessary to estimate the urea excreted during the period. Each gram of urea nitrogen represents, approximately, 6.25 gm. of protein, which, in turn, represents an O_2 consumption of 6.25×0.957 , or 5.92 litres, and a CO_2 output of 4.75 litres. Knowing the amount of protein metabolised during the experiment, its O_2 and CO_2 equivalents are subtracted from the values for the total O_2 used and CO_2 liberated, thus enabling the *non-protein* respiratory quotient to be calculated. The relationship between the non-protein r.q., the O_2 used in combustion of carbohydrate and fat, and the heat output, is given in the conversion tables compiled by Lusk and by McClendon, an abridged form of which is shown.

Non-protein R.Q.	Oxygen equivalent per litre.		Kilocalcs.
	Carbohydrate, in gm.	Fat, in gm.	
0.707	0.000	0.502	4.686
0.75	0.173	0.433	4.739
0.80	0.375	0.350	4.801
0.85	0.580	0.267	4.862
0.90	0.793	0.180	4.924
0.95	1.010	0.091	4.985
1.00	1.232	0.000	5.047

Thus, if analysis of O_2 and CO_2 , and subtraction of the values for protein metabolised during the period, gives a non-protein r.q. of 0.85, each litre of O_2 used in non-protein combustion represents the oxidation of a mixture of 0.580 gm. of carbohydrate and 0.267 gm. of fat, and corresponds to a heat output of 4.862 kilocalcs. According to Poulton (1937), the indirect method of calculating heat output from oxygen consumption is untrustworthy, since some of the O_2 is being used for constructive metabolism. The non-protein CO_2 , however, comes entirely from the combustion of carbohydrate and fat, and its output should be measured in preference to the O_2 intake.

Energy Output of the Organism.—The growing animal or plant uses much of its available energy in constructive endergonic reactions involving the building-up of tissues and storage products. In higher animals at constant weight, more than 90 per cent. of the energy released by oxidation may escape as heat, the remainder being spent in mechanical work by the muscles, and osmotic work by the secreting glands, including the kidneys. The body-temperature of man and the higher vertebrates is kept poised about a constant value that represents the most favourable heat-production level for the co-ordinated working to the entire organism. This value is 98.4° F. (36° C.), for man ; rising to about 106° F. for birds.

Expressed as percentages of total loss during rest or slight activity, heat is transferred from the human body in the following ways : *lungs*, warming expired air, 3.5 ; *lungs*, by evaporation, 7.2 ; *kidneys* and *intestine*, warming excreted material, 1.3 ; *skin*, evaporation of sweat, 14.5 ; *skin*, by convection, conduction and radiation, 73. Most of the heat lost is by convection currents from the surface of the body and its coverings. Human surface-temperature ranges from 29° C. (feet) to 35° C. (head and neck), in an ordinary environment of about 24° C. The body surface covered with clothes has a temperature of 33° C.—35° C.

According to Markham (1942), the ideal climate, in which men neither shiver nor perspire excessively when at rest, has a temperature

range of 60° to 76° F., with a relative humidity varying from 40 to 70 per cent.

Basal Metabolism.—The minimum energy-turnover capable of maintaining life is termed the basal metabolism, and is defined as the heat output of a subject, awake, but in a state of maximum bodily and mental rest, 12–18 hours after a meal. It is assumed to represent the continuous metabolic basis of existence, on which is superimposed all the metabolic responses to the demands of daily activity. The basal metabolism rate (*b.m.r.*), is found clinically from the respiration rate, measured by indirect calorimetry of the recumbent, resting subject. It is usually expressed as a percentage above or below the theoretical normal standard for a subject of the same age, sex, height and weight.

Since heat is lost chiefly from the surface, the *b.m.* rate depends on the surface area of the body. This is approximately 1.8 sq. metres for the average adult, and may be calculated from the DuBois formula :

$$S = W^a \times H^b \times 71.84,$$

where *S* is surface area, in sq. cm. ; *W* is body-weight, in kgm. ; *H* is height, in cm. ; *a* is a constant, 0.425 ; and *b* is a constant, 0.725.

The calculation is simplified by the use of nomograms and tables compiled by Hawk and Bergeim ("Practical Physiological Chemistry," 1937) ; DuBois ("Basal Metabolism," 1936) ; Cantarow and Trumper, "Clinical Biochemistry," 1940).

Average values, in terms of *b.m.r.*, in kilocal. per sq. metre of body surface per hour, are : adult males, 40 ; adult females, 37 ; children, 57 (six years), 50 (twelve years). The rate falls progressively in both sexes over fifty years of age. In terms of adult body-weight, the *b.m.r.* is, roughly, 1 kilocal. per kgm. per hour, or 1,680 kilocal. for a 70 kgm. man, in twenty-four hours. This indicates that half of the ordinary energy requirement is expended in the maintenance of basal metabolism, the overhead charge imposed by Nature on all human existence.

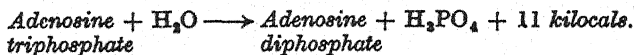
Chemical factors affecting *b.m.r.* include the hormones of the anterior pituitary, adrenal and thyroid glands, injection or hypersecretion of which increase the rate, which, in severe exophthalmic goitre, may rise to 100 per cent. above the normal value. Thyroid deficiency, as in myxedema, may reduce the rate to 60 per cent. of the normal. Hence, *b.m.r.* estimations are of use in the differential diagnosis of thyroid disturbances.

Other pyrogenic agents capable of raising temperature and *b.m.* rate are 4 : 6-dinitrophenols and the fever toxins. By violent muscular work, oxygen consumption, from the normal level of 200–250 ml. per minute, may be raised to 3–4 litres, with a consequent sixteen-fold increase in total metabolism. Each rise of 1° F. in body temperature corresponds to an increase of about 7 per cent. in the basal metabolic rate.

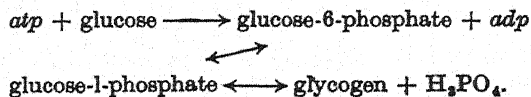
Specific Dynamic Effect of Nutrients.—A meal of 25 gm. of protein yields, in theory, 100 kilocal. ; actually, as found by calorimetry, the yield is nearly 130 kilocal., showing that metabolism of the protein has necessitated the release of 30 additional kilocal. from other sources. This *specific dynamic* effect or action is displayed also by the amino acids, notably glycine and alanine, and by ammonium salts. It is attributed to the oxidation of carbohydrate to provide energy for the synthesis of urea in the last stage of protein metabolism (Lundsgaard, 1942). Carbohydrates and fats show a slight dynamic effect, which is not more than a 4 per cent. increase of their theoretical heat-value, and is due, probably, to surplus energy released for the requirements of digestion and absorption. The ordinary mixed diet has a dynamic effect of about 12 per cent., which indicates that the maintenance of resting equilibrium requires a calorie intake of 12 per cent. above that needed in theory for the maintenance of the basal metabolic rate.

Mechanism of Energy Exchange.—Energy released by exergonic reactions in the tissues is not entirely converted into heat or partially used for endergonic construction reactions; some of it may be accumulated in the energy-rich phosphate-bond systems, such as —N—P—, in phosphocreatine, —C—O—P—, in phosphopyruvate, and —P—O—P—, in adenosine polyphosphates.

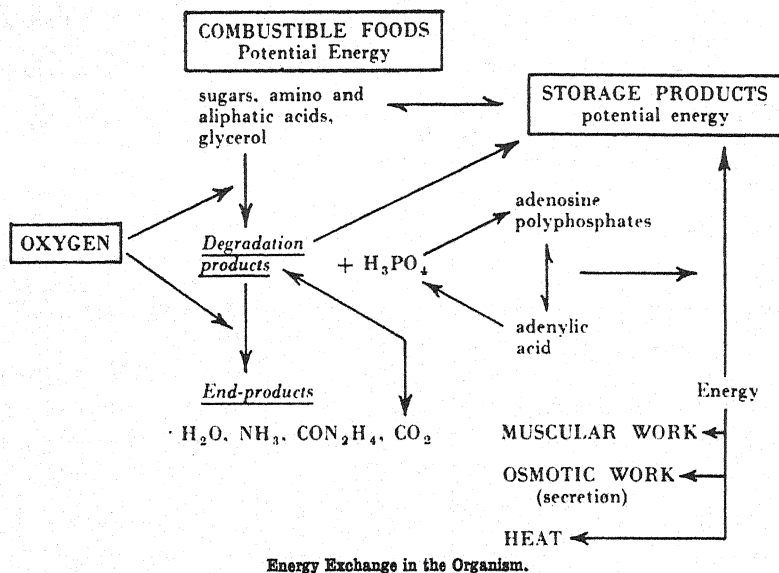
These energy-rich phosphate-bond compounds yield about 11 kilocal. per gm. molecule, on hydrolysis, and are quite distinct from the ordinary phosphate esters, such as glucose-1-phosphate and glycerophosphate, the linkage of which is poor in energy, and only yields some 3 kilocal., on hydrolysis. In adenosine triphosphate, the first ester linkage is an ordinary bond, the two terminal phosphate groups in the chain carry the energy, and maintain a shuttle-service of alternate dephosphorylation and phosphorylation:



Phosphate-bond energy is generated by dehydrogenation reactions, such as the conversion of phospho-2-glycerate into phosphopyruvate (p. 334). The energy thus concentrated is transferred usually to adenylic acid, raising it successively to adenosine diphosphate (*adp.*) and triphosphate (*atp*), and forming a pool that provides energy for sugar phosphorylation, muscle contraction, and other reactions. Following the scheme proposed by Cori, and accepted by Lipmann (1941), the formation of glycogen from glucose is represented as :—



The first change is endergonic, and involves a loss of 8 kilocal. used in converting glucose into a phosphate; the other reactions involve very little energy change, and can be reversed by changing the concentration of the reactants.



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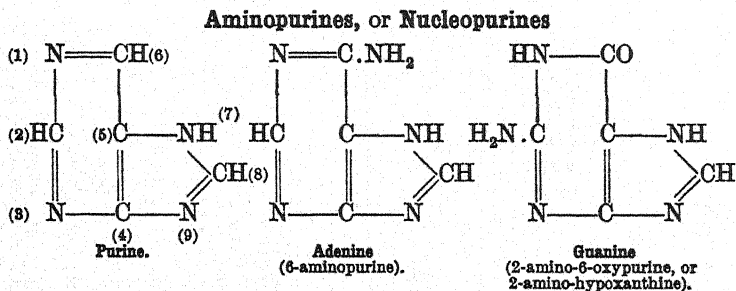
CHAPTER 20

PURINES AND PYRIMIDINES

Purines.—The biological purines are simple hydroxy, amino, or methyl derivatives of a parent purine ring, which does not occur free in nature. Hydroxy purines, *hypoxanthine* and *xanthine*, occur in tissues and tissue fluids of many animals; *uric acid*, a trihydroxy purine, is the chief nitrogenous excretory product of birds and snakes, and is invariably present in mammalian urine.

Amino purines, represented by *adenine* and *guanine*, are components of nucleic acid and the nucleosides. Methyl purines are the characteristic alkaloids of tea, coffee, and cocoa.

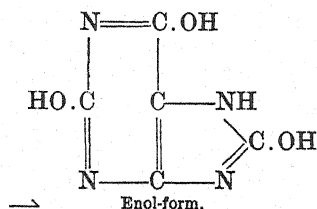
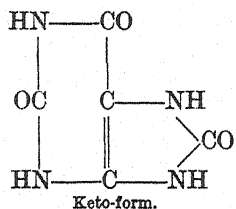
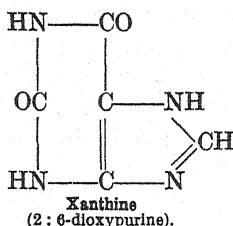
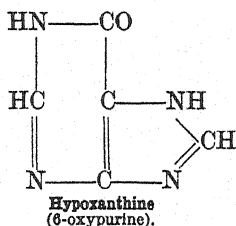
Sources of the Purines.—The hydroxy and amino purines are derived chiefly from the nucleoproteins of the diet, although there is evidence that the organism can synthesise the purine ring from the amino acid histidine. Nucleoproteins are compound proteins, the non-protein being nucleic acid, which is liberated during alimentary digestion. Nucleic acid is composed of four nucleotide units, each of which is a phosphoric ester of a nucleoside, or compound of a pentose and an aminopurine or a pyrimidine (p. 142).



Adenine, $\text{C}_5\text{H}_5\text{N}_5$, the simpler of the nucleopurines, is widely distributed in plant and animal nucleoprotein, in the mononucleoside *adenosine*, found in animal tissues, as polyphosphates in muscle, and as mononucleosides in beet, and human blood and urine. Adenine occurs in colourless needles that are slightly soluble in cold water, but freely soluble in acids or alkalies. Large amounts of adenine may appear in the urine in conditions of leukaemia. On deamination by enzymes or by nitrous acid, adenine is converted into the corresponding 6-oxypurine, *hypoxanthine*.

Guanine, $C_5H_5N_5O$, is widespread as a nucleopurine, and often accompanies hypoxanthine in plants. It occurs in guano, in muscle, in the juice of the beet, in many leguminous seeds, and as an articular deposit in the joints of swine suffering from guanine gout. It resembles adenine, but is a stronger base. On oxidation, it gives rise to *guanidine*, $H_2H-C(:NH)-NH_2$, which serves to distinguish it from adenine and all other purines. On deamination, guanine is converted into the corresponding 2 : 6-dioxypurine, *xanthine*.

Hydroxy-, or Oxypurines



Uric Acid

(2 : 6 : 8-trioxypurine).

Each of these purines can exist either in keto or in enol form, by tautomeric change : $-\text{NH} \cdot \text{CO}- \rightleftharpoons -\text{N}=\text{C}(\text{OH})-$.

Hypoxanthine, $C_5H_4N_4O$, has been obtained chiefly from nucleotides. It is present in extracts of glandular and muscle tissue, in fish sperm and bone marrow, and, in traces, in milk and urine. Urinary hypoxanthine is greatly increased in leukæmia. On oxidation, it is converted into xanthine.

Xanthine, $C_5H_4N_4O_2$, is present, alone or combined, in many animal extracts, and is one of the minor constituents in mammalian urine, and in guano. Like hypoxanthine, it is chiefly of interest in being a deamination product of an amino purine, and an obligatory intermediate product in uric acid metabolism.

Paraxanthine, 1 : 7-dimethyl xanthine, is concerned in basal metabolism, and inhibits the effect of the thyroid hormone. It is present in small amounts in urine.

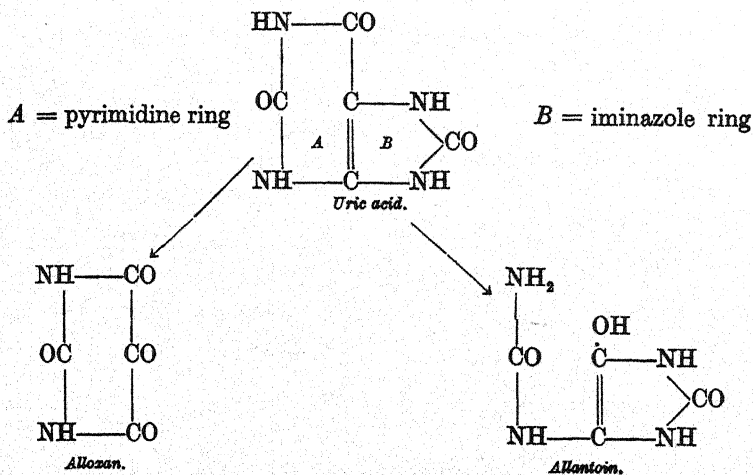
Uric acid, $C_5H_4N_4O_3$, the most important of the oxidised purines, is the chief end-product of purine metabolism in man and the higher apes. It is the chief end-product of protein metabolism in uricotelic animals, birds and snakes, and some invertebrates. It is the least soluble of all the forms in which nitrogen is excreted, and appears in calculi, articular deposits, and urinary sediments.

Distribution of Uric Acid in the Human Body.—Blood contains 1–5 mg. per 100 ml. The value is raised typically in gout, lead poisoning, leukaemia, and renal inefficiency. The uric acid content of the tissues is usually lower than that of the blood, unless in a region of active purine metabolism.

Urine contains about 40–150 mg. uric acid per 100 ml., representing the average daily excretion of 0.5–1.5 gm. The value is extremely variable, depending on dietetic and individual conditions.

Properties of Uric Acid.—The acid is colourless, odourless, and tasteless. Its solubility in water is extremely low, being 1 : 39,000 at 18° C., and 1 : 15,500 at body temperature (37° C.). It dissolves readily in alkalis with the formation of (i.) acid, or monobasic urates, and (ii.) neutral, or dibasic urates. Biological urates are acid urates; the neutral urates are only stable under conditions of alkalinity not found in the organism. Although uric acid is not a true organic acid, since it contains no carboxyl groups, it can form stable salts by enolisation of the three hydroxyl groups, as displayed in the form $C_5HN_4(OH)_3$.

Oxidation of Uric Acid.—Oxidation of uric acid in alkaline solution opens up the pyrimidine ring, producing *allantoin*. Oxidation in acid solution opens up the iminazole ring, producing *alloxan*.



Allantoin is not a purine, since the characteristic double ring has been opened, but is of importance since it represents the end-product of purine metabolism in many animals. It occurs in the allantoinic fluid of herbivora, and in the urine of herbivora and carnivora in the following average concentrations; expressed in grams per litre: calf, 5-6; cow, 2-2.5; sheep, horse, pig, rabbit, 0.7-1.8; dog, 1.9-2.6. The amount excreted daily by the cow is 20-30 gm. Only small and variable traces are found in human urine; its place being taken by its precursor uric acid.

Allantoin is much more soluble than uric acid, the solubility in water being 1:160 at 20° C. It is much more desirable as an end-product of purine metabolism, since it does not form calculi.

Alloxan is chiefly of chemical interest in regard to the structure of uric acid. Injected into the animal, it destroys the islet tissue of the pancreas, and leads to diabetes (Shaw Dunn, 1943).

Reactions of Uric Acid.—In uric acid, but not in the other natural oxypurines or aminopurines, the H atom at position 7 is very labile to alkalis, and uric acid in dilute sodium carbonate solution can reduce Ag^+ to black colloidal silver (Schiff's test), and phosphotungstate or phosphomolybdate to a deep blue pigment (Folin's test), and gives a bright yellow with dichloroquinone-chloroimide. These tests are of use in the colorimetric estimation of uric acid.

Murexide Test.—This reaction is very useful for detecting uric acid in solids, such as powdered calculi. The solid is moistened with concentrated nitric acid, and dried in a dish on a water bath. The warm residue has a brilliant orange-carmine colour if uric acid be present. Addition of an alkali changes the colour to purple (ammonium hydroxide), or violet (sodium hydroxide). Other purines, notably xanthine, guanine, and caffeine, give somewhat similar colours, but they do not occur in urinary precipitates and calculi.

Natural Purine Derivatives

Purines occur as (i.) nucleosides, (ii.) mononucleotides, and (iii.) polynucleotides, or nucleic acids. Nucleosides are glycosides of a purine or pyrimidine base. Nucleotides are phosphoric esters of nucleosides, in which the phosphoric radicle is joined to the third or fifth carbon in the sugar residue.

(i.) *Purine Nucleosides* :—

Adenosine, 7-adenine-D-riboside.

Guanosine, 7-guanine-D-riboside.

Inosine, 7-hypoxanthine-D-riboside.

(ii.) *Purine Mononucleotides* :—

Adenylic acid (from muscle), adenine-5-phosphoriboside.

Adenylic acid (from yeast), adenine-3-phosphoriboside.

Inosinic acid, hypoxanthine-5-phosphoriboside.

Guanylic acid, guanosine phosphate.

(iii.) *Nucleotide Derivatives* :—

Adenosine triphosphoric acid (adenyl pyrophosphate).

Adenosine diphosphoric acid (adenyl phosphate).

Nicotinamide-adenine dinucleotides (*NAD*), namely co-enzyme I and co-enzyme II.

Riboflavin-adenine-dinucleotide.

(iv.) *Polynucleotides* :—

Thymo-nucleic acid, desoxyribose nucleic acid, from cell nuclei of both plants and animals.

Yeast-nucleic acid, ribose nucleic acid, from extra-nuclear cytoplasm of plants and animals.

The nucleosides are prepared by alkaline hydrolysis of the parent nucleotides, and rarely occur in a free state.

Mononucleotides

Adenylic acid, now called "muscle adenylic acid" to distinguish it from adenylic acid obtained from yeast, is widely distributed in animal tissue, and ranks along with histamine and acetyl choline as a powerful vaso-dilator. On deamination it is converted to the much less active inosinic acid. Adenylic diphosphate (or pyrophosphate) is found in muscle where it acts as a donator of phosphoric acid in the contraction process (p. 332), becoming degraded to adenyl phosphate during the change.

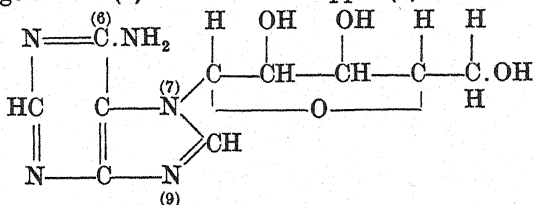
Co-enzyme I is a diphospho-pyridine nucleotide of adenine. Co-enzyme II (Warburg) is a triphospho-pyridine nucleotide of adenine. Both co-enzymes act as hydrogen carriers in respiration and can unite with specific proteins to form various enzyme systems. The co-enzyme of D-amino acid oxidase is a diphospho-riboflavin nucleotide of adenine.

Polynucleotides

Nucleic acids occur in union with protamines or histones as the nucleo-proteins found in all plants and animals. They are substances of high molecular weight, the simplest unit being a tetranucleotide containing four different basic residues (adenine, guanine, cytosine, and thymine or uracil). On hydrolysis, a tetranucleotide yields four different nucleotides, each of which is changed into a nucleoside by alkaline hydrolysis. The sugar residue in the nucleic acid depends on the type of acid, and may be D-ribose or 2-desoxyribose. From these facts, provisional type formulæ have been assigned to the nucleic acids (p. 142).

Feulgen's Test for Nucleic Acid.—The material is hydrolysed with N/10 HCl at 60° C. for four minutes, and then treated with Schiff's aldehyde reagent (a 1 per cent. solution of rosaniline decolorised with SO₂), a red colour develops if animal or thymo-nucleic acid be present. The test depends on the presence of desoxyribose.

Structure of the Nucleosides.—The constitution assigned to the important nucleotides is given elsewhere in connection with their functions. The formula of a typical nucleoside is shown in adenosine. There is some uncertainty as to the point of attachment of the ribose group, and it is sometimes represented in union with the lower nitrogen atom (9) instead of the upper (7).



Adenosine

Phosphorylation of the terminal —CH₂.OH in the ribose group produces muscle adenylic acid, from which in turn are derived adenylic phosphate and adenylic pyrophosphate (adenosine triphosphate). Adenosine triphosphate (*ATP*) is the chief phosphorylating agent in the organism, and, when activated by the appropriate tissue enzyme, can contribute PO₄ to creatine (yielding phosphocreatine), and to many intermediates in sugar metabolism. Activated by myosin, it provides energy for muscle contraction.

According to Conway (1938), adenosine and adenylic acid are the chief sources of the ammonia latent in blood, and are deaminated by plasma and tissue deaminases, with loss of the free amino group at position (6).

Plant Purines.—While all plants are able to synthesise purines, many do so in great excess of their nuclear requirements.

Purine Content of Plants

Expressed as mg. Uric Acid per 100 gm. Fresh Edible Material

Plant.	Uric Acid.	Plant.	Uric Acid.
Lentil . . .	160	Mushroom . .	15-30
Green pea . .	54-80	Celery . . .	15
Spinach . . .	70	Radish . . .	15
Kidney bean .	51	French bean .	6
Cauliflower .	24	Lettuce . . .	6
Asparagus . .	24	Potato . . .	6

Although these average values only refer to the edible portions of the plant, and do not discriminate between the various forms of purine present, they indicate the existence of a group of vegetables rich in purine, and represented by the *Leguminosæ*. In addition, another class exists rich in methylated purines, represented by tea and coffee, and other natural sources of the purine alkaloids.

Transformation and Degradation of Purine in Animals.—Animals other than birds and reptiles excrete their waste protein nitrogen as urea, and are said to be *ureotelic*. In ureotelic animals, purine metabolism proceeds along independent lines. The purines of the diet, chiefly nucleotides and nucleosides liberated from nucleoproteins, are resolved into their constituent amino purines by the enzymes of the alimentary tract and mucosa. The amino purines are absorbed into the portal system, and, if not utilised, are deaminated by the appropriate enzymes, adenase and guanase, found in the liver.

Adenine is converted into hypoxanthine, and guanine is converted into xanthine. Subsequently, by the action of xanthine oxidase both hypoxanthine and xanthine are converted into uric acid. Here the transformation stops in man, the higher apes, the Dalmatian dog, and the birds and reptiles; in consequence, uric acid is the characteristic end-product of purine metabolism. Most of the higher animals, however, are able to oxidise uric acid, and by opening part of the purine ring convert it into the much more soluble compound, allantoin, which is readily eliminated as a urinary solute.

Summary of Purine Transformation in Animals

Alimentary tract	<p>Nucleoprotein ↓ Nucleic acid ↓ Nucleotide ↓ Nucleosides</p>
Liver and other tissues	<p> <div style="display: flex; justify-content: space-around;"> <div> <p>Adenine ↓ Hypoxanthine ↓ Allantoin</p> </div> <div> <p>Guanine ↓ Xanthine ↓ Uric acid</p> </div> </div> <p style="text-align: center;">Hypoxanthine → Xanthine</p> </p>
Urine	<p>Allantoin ← Uric acid</p>

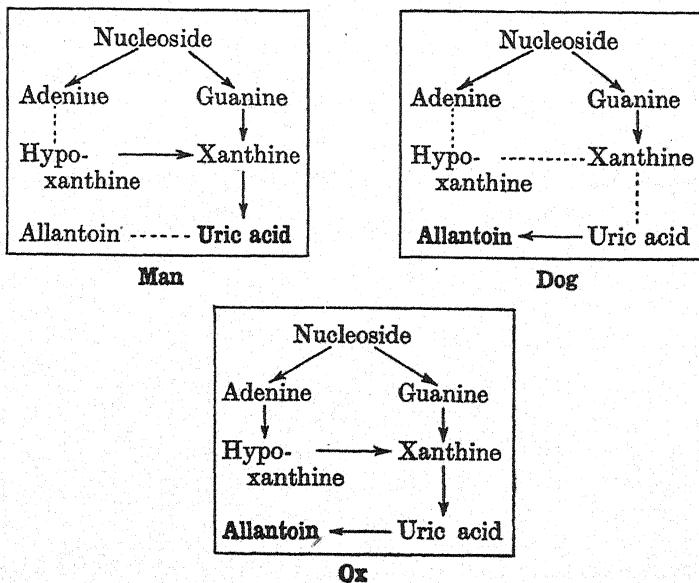
Enzymes concerned in Purine Metabolism.—(i.) **Adenase**, or adenine deaminase, is relatively rare. It is found in the spleen and liver of the pig, and in the liver of the ox, but is absent from the liver of man, the dog, and the rabbit.

(ii.) **Guanase**, or guanine deaminase, is widely distributed, and is present in the liver and other tissues of most mammals, the pig being a remarkable exception.

(iii.) **Xanthine oxidase** catalyses the oxidation of both hypoxanthine and xanthine to uric acid, and also attacks adenine. It is found in the liver of many mammals, but not in the dog or the rat, indicating that in these animals uric acid must have an extra-hepatic source. Xanthine oxidase is a constant constituent of cow's milk.

(iv.) **Uricase**, or uric acid oxidase, is claimed to occur in the livers of all mammals except man and the higher apes. It is a purinoclastic enzyme, and converts uric acid into the non-purine allantoin.

Diagram of enzyme distribution in mammalian liver



The continuous line indicates that the appropriate enzyme is present in the liver; the dotted line shows that it is absent. End-products of metabolism are in heavy type.

The diagram is based on the work of Jones, and, although the absolute specificity of the enzymes is not fully established, it

explains the occurrence of the different end-products in the different species.

Destruction of Uric Acid.—Man and the higher apes have very little power of destroying uric acid compared with that possessed by other mammals, and hence it is the chief end-product of purine metabolism. Folin (1924) reports that 30–70 per cent. of injected uric acid may be destroyed or transformed by the human organism, the nature of the change being obscure. In the dog, at least, the liver is the principal site of uric acid destruction, as shown by the effect of complete hepatectomy, which leads to the appearance of large quantities of uric acid, instead of allantoin, in the urine.

The Uricolytic Index.—This is believed to represent the ability of the animal to destroy uric acid, and is expressed as the percentage ratio of the allantoin nitrogen to the sum of allantoin and uric acid nitrogen excreted.

$$\text{Uricolytic Index} = \frac{\text{Allantoin N} \times 100}{\text{Allantoin N} + \text{Uric acid N}}$$

Among carnivora, the low index of the Dalmatian dog is of note, this animal having a uric acid excretion almost as high as that of man. Onslow has shown that the high index is a dominant Mendelian character, and when Dalmatian dogs are crossed with other dogs, the offspring display the uricolytic efficiency of the majority of the carnivora.

Representative uricolytic indices are: Man, 0; chimpanzee, 0; monkey, 89; cow, 93; pig, cat, rabbit, 96; dog, 98 (Dalmatian dog, 32); rat, 96.

Purine Metabolism.—On a mixed dietary, the average human renal output of purine, *per diem*, is 0.6–0.9 gm. as uric acid, 30–50 mg. of purine bases (adenine, guanine hypoxanthine and xanthine), and a variable amount of methyl purines, derived from caffeine and theobromine. This output corresponds to a uric acid level in the blood of 1.5–3.5 mg. per 100 ml., a value which may be raised tenfold in conditions of renal incompetence, gout, lead-poisoning, and azotæmic nephritis. The intestinal excretion of purine is hard to assess owing to the bacterial destruction of these compounds. At least half the purine output is exogenous, being derived from the nucleoproteins of the dietary, and may be increased considerably by the ingestion of foodstuffs rich in nuclear material, such as sweetbread (pancreas or thymus), liver and kidney.

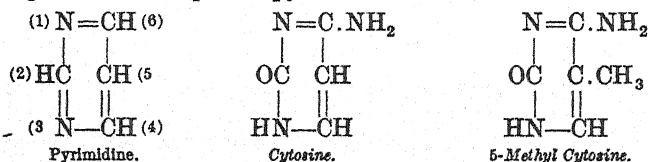
The human organism has the power of synthesising purines, as is shown by the fact that milk, an adequate food for the growing child, is almost purine-free. The precursors of these purines,

which are elaborated to provide nucleoproteins for the cells and tissues, appear to be the amino acids, arginine and histidine.

On a purine-free diet of starch and cream, Folin was able to reduce his uric acid output to 0.3 gm. *per diem*, approximately. The experiment was not continued for more than a few days, and this value probably represents the maximal level of the *endogenous* metabolism of purines derived from non-purine sources within the organism. Endogenous uric acid arises from the nucleic acid liberated by the destruction of cell nuclei, especially those of the leucocytes, which are being continually manufactured and broken down, and the erythrocytes, which are denucleated prior to being issued into the general circulation. Any condition of hyperleucæmia or of increased leucocyte destruction, tends to raise the output of endogenous uric acid. The hæmopoietic, or blood-forming apparatus appears to be the chief source of endogenous purine, but, in addition, it is probable that some uric acid is derived from the adenylyl and other nucleotides that participate in carbohydrate metabolism.

PYRIMIDINES

At least four pyrimidine derivatives occur in nucleoproteins, each being referred to a parent pyrimidine not found free in nature.



Like the aminopurines, the aminopyrimidines occur as components of nucleosides, and are liberated as end-products of nucleoprotein digestion. By deamination, *cytosine* (2-oxy-6-amino pyrimidine) is converted to *uracil* (2 : 6-dioxy pyrimidine); and 5-methyl cytosine is converted to *thymine* (2 : 6-dioxy-5-methyl pyrimidine), the change being analogous to the deamination of adenine and guanine.

Thymine was originally obtained as an end-product of the hydrolysis of thymo-nucleic acid, its precursor in the nucleoside is 5-methyl cytosine.

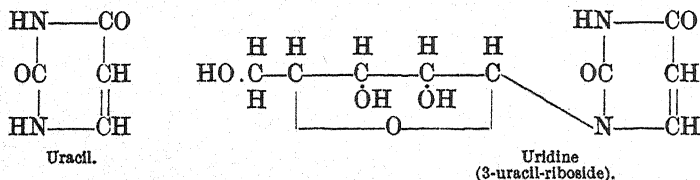
Pyrimidine Derivatives

Pyrimidine nucleosides of cytosine, uracil, thymine, methyl cytosine are obtained as products of nucleic acid hydrolysis. *Vicine*, a D-glucoside of 2, 5-diamino-4, 6-dihydroxy pyrimidine occurs in plants.

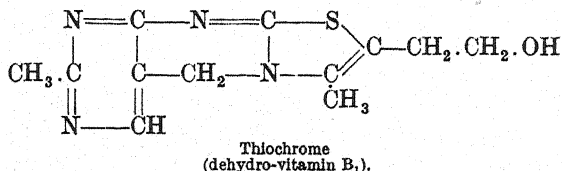
Uridine, uracil nucleoside, is obtained from *uracil nucleotide*, which occurs along with *cytosine nucleotide* in yeast-nucleic acid.

Thymo-nucleic acid contains cytosine and 5-methyl cytosine nucleotides as its pyrimidine components.

In structure, these nucleotides correspond to the purine nucleotides in being phosphoric esters of nitrogenous glycosides.



Pyrimidine Vitamins.—The pyrimidine ring occurs in vitamin B₁, thiamine, and in vitamin B₂, riboflavin (pp. 226, 285), and in the co-enzyme, co-carboxylase, which is a phosphoric ester of thiamine (p. 282). Oxidation of thiamine leads to the formation of *thiochrome*, a pigment found in yeast, and characterised by its fluorescence in ultra-violet light.



Pyrimidine Nucleotides.—Yeast nucleic acid contains two pyrimidine nucleotides, *uracil nucleotide* and *cytosine nucleotide*; thymus nucleic acid contains *cytosine nucleotide* and *5-methyl cytosine nucleotide*. In structure, these nucleotides correspond to the purine nucleotides, being phosphoric esters of nucleosides, or compounds of pentose and a nitrogenous base. Thus, *uridine* is the nucleoside derived from uracil.

Pyrimidines are very reactive, and readily undergo reversible oxidation, which suggests that they participate in the intense metabolism that is characteristic of nuclear tissues.

Uracil and thymine when fed to dogs are excreted as urea. Cytosine is partly deaminated to uracil, and partly excreted unchanged.

Nucleoprotein Viruses.—Nucleic acids show characteristic light absorption in the region 260 mμ due to the purine and pyrimidine units in the molecule. Chromatin and viruses show a similar absorption, owing to their nucleoprotein construction. These viruses range in size from *vaccinia*, which evokes cow-pox, and is probably the smallpox virus modified. *Vaccinia* has a diameter of 175–225 mμ, and m.wt. 4.3 × 10⁹, and resembles a bacterium.

Tobacco mosaic virus, as shown by electron-microscopy, is a rod, 180 μ by 15 μ , m.wt. $17-40 \times 10^5$. Foot and mouth disease virus has a diameter of 10 μ and m.wt. 4×10^5 , and may approximate to the minimum size limit possible for the self-reproduction that characterises viruses and genes when occupying living cells.

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CHAPTER-21

NITROGENOUS BASES

THE term *simpler natural bases* has been applied by Barger to describe a type of nitrogen compounds widely distributed in plants and animals. Many are derived from the natural amino acids, and many have a powerful physiological action. As a group, all are water-soluble and precipitated by phosphotungstic acid.

Classification of the Simpler Bases.—(1) *Amines* derived from natural amino acids. Proteinogenous amines.

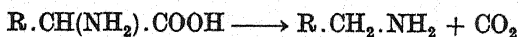
(2) *Betaines*, onium derivatives of methyl amino acids.

(3) *Cholines*, onium derivatives of methyl amino alcohols.

(4) *Guanidines*, derivatives of the base $\text{H}_2\text{N} \cdot \text{C}(\text{NH}) \cdot \text{NH}_2$.

(5) *Miscellaneous bases*.

(1) **Proteinogenous Amines.**—An α -amino acid can give rise to a corresponding amine by decarboxylation, in accordance with the type formula :—



Aminogenic organisms are constant inhabitants of the human intestine, and decarboxylation may be regarded as a by-path in amino acid catabolism. It is probable, however, that most of these autogenous amines are transformed or destroyed before being absorbed, otherwise, a large protein meal would be followed by frequent instead of occasional discomfort.

Sympathomimetic or Pressor Amines.—Amines may be classified in terms of their physiological action into *pressor* and *depressor* amines. The pressors being termed *sympathomimetic* because their action resembles certain effects got by stimulation of the sympathetic nervous system, namely :—

(1) Vascular constriction with accompanying rise in blood pressure.

(2) Increased force of cardiac contraction.

(3) Inhibition of peristaltic movements in the alimentary tract.

(4) Dilatation of the pupil of the eye.

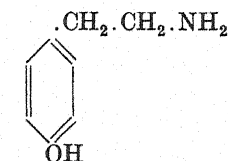
(5) Contraction of the uterus.

Primary amines are slightly sympathomimetic, the effect being maximal in *n*-hexylamine, $\text{C}_6\text{H}_{13} \cdot \text{NH}_2$, but the principal pressor bases are the complex amines, tyramine and tryptamine, as well as the hormone, adrenaline.

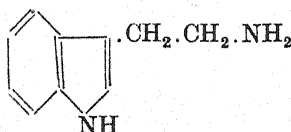
Tyramine, *p*-hydroxy phenylethylamine, $\text{HO.C}_6\text{H}_4.\text{CH}_2.\text{CH}_2.\text{NH}_2$, the amine derived from tyrosine, is the chief pressor base found in some extracts of ergot, in putrefied animal tissues, and in "ripe" cheese.

Pharmacologically, tyramine has a weaker, slower, more persistent and less toxic action than adrenaline. Administered orally or intravenously it causes a rise in blood pressure and an increase in the force of the heart beat. It is also an *ecbolic*, causing contraction of the pregnant uterus, but, as some assert, inhibiting the contraction of the non-pregnant uterus. Tyramine gives the characteristic colour tests for tyrosine (p. 165).

Tryptamine, β -indolyl ethylamine, the amine corresponding to tryptophane, resembles tyramine, but is less powerful. It has been obtained as a product of protein putrefaction, but has not yet been found free in plants.



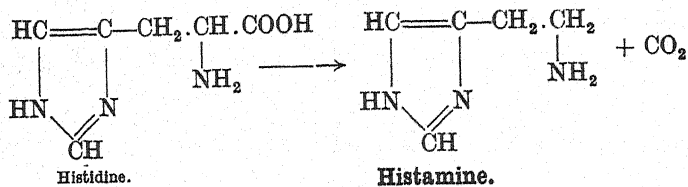
Tyramine.



Tryptamine.

Depressor Amines.—*Histamine*, β -iminazolyl-ethylamine, the amine derived from histidine, is one of the chief principles of ergot, in which it was discovered by Barger and Dale (1910). It has been obtained also by the putrefaction of histidine and proteins containing histidine. Its chief animal sources are the aqueous or alcoholic extract of lung, liver, spleen, intestinal mucosa, and the posterior lobe of the pituitary gland.

For many years it has been known that simple extracts of animal tissues, when injected intravenously, may cause a profound fall in blood pressure in experimental animals, especially carnivora. This depressor effect is essentially a vaso-dilatation, although cardiac and respiratory phenomena may be involved also. At one time it was believed that the depressor effect was due to choline, but this was disproved when Vincent and others showed that the effect was not inhibited by a previous injection of atropine, the pharmacological antagonist of choline. The histamine is not derived from infective or post-mortem changes in the tissues, as it can be obtained when living lung tissue is taken from an anaesthetised animal, and at once frozen in alcohol, which indicates that the amine is immediately available in the living cell, probably as part of the system :—



Detection of Histamine.—The physiological tests for histamine are : (i.) contraction of isolated uterus or intestine of guinea-pig or rat ; (ii.) lowering of blood pressure after injection into cats or dogs ; (iii.) dermographic response when injected subcutaneously into human subjects ; (iv.) bronchospasm when injected into guinea-pigs. Various colour reactions are also used.

Physiological Properties of Histamine.—This amine is sharply differentiated from other proteinogenous amines by its powerful depressor effect. Intravenous injection is followed by a quick fall in blood pressure, which is not inhibited by atropine. This is due to a direct action on the capillary wall, causing paralysis, loss of tone, passive dilatation, and increased permeability.

The capillary dilator effect is accompanied by a weaker constrictor action on the arterioles which is insufficient to check general capillary engorgement ; consequently, histamine injection or liberation causes a rapid and often fatal fall in blood pressure. This effect is most marked in carnivora, and may be weak or lacking in anaesthetised rodents, a paradox ascribed to the desensitising effect of many anaesthetics.

Although histamine injection evokes a typical "shock" effect, not all types of surgical and other "shock" can be due to its release, since anaesthetised men and unanaesthetised cats can cope with relatively large amounts of histamine, without exhibiting shock. Histamine is easily released from injured or burnt tissues, but the amount normally free in the body is very small. In many forms of shock blood histamine is not increased. Histamine is liberated in the skin, even after mild stimulation not involving cellular damage. Applied to raw surfaces or injected intra-dermally, it causes pain, and may be the actual chemical mediator in cutaneous pain sensation (Rosenthal and Minard, 1939).

Histamine stimulates involuntary muscle, such as that of the intestinal tract, uterus, bronchioles and arterioles, the response depends on species and on physiological circumstances, and may lead to fatal asphyxia in some animals, notably the young guinea-pig.

Histamine is a powerful secretagogue, and evokes a rapid flow of gastric juice and saliva when injected into the human subject.

In response to stimulation by food, gastric mucosa secretes a hormone, *gastrin*, which, in turn, evokes the flow of more juice. Gastrin closely resembles histamine, and may be the actual amine.

Putrescine, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$, tetramethylene diamine, is derived from the diamino acid ornithine, by decarboxylation.

Cadaverine, $\text{H}_2\text{N} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NH}_2$, pentamethylene diamine, is derived from lysine, by decarboxylation. These diamines were formerly regarded as being typical "ptomaines," or poisonous bases produced by the bacterial decomposition of proteins. They are not highly toxic compounds, and have been detected in the urine.

Spermine, $\text{H}_2\text{N} \cdot (\text{CH}_2)_3 \cdot \text{NH} \cdot (\text{CH}_2)_4 \cdot \text{NH} \cdot (\text{CH}_2)_3 \cdot \text{NH}_2$, and *Spermidine*, $\text{H}_2\text{N} \cdot (\text{CH}_2)_3 \cdot \text{NH} \cdot (\text{CH}_2)_4 \cdot \text{NH}_2$, are polyamines isolated and identified by Rosenheim. They occur in extracts of testicle and other tissues.

Spermine is widely distributed in animal tissues, and is manufactured or stored in the prostate gland. Representative values, in mg. per 100 gm. fresh tissue, are: human prostate, 130; human pancreas, 16; ox pancreas, 25-30; human liver, 10; testicle, spleen, kidney, 1-7.

The distribution of these and other amines suggests that decarboxylation can take place in many different tissues.

Amines not Directly Derived from Amino Acids.—Some of the primary amines found in tissues cannot be referred to any known parent amino acid, and must be formed by special synthesis. Among them are:—

Sphingosine, a primary amine derived from an unsaturated 18-carbon acid. It is a constituent of the cerebrosides or glycolipides (p. 183).

Guanidines.—This group includes guanidine, or imino urea, $\text{H}_2\text{N} \cdot \text{C}(\text{:NH}) \cdot \text{NH}_2$, and its derivatives. Guanidine occurs in the seedlings of the vetch after germination, but has not been detected as a free base in the animal body. It is toxic, and on injection evokes spasms closely resembling those of hypocalcæmic tetany. Guanidine is readily obtained by the oxidation of proteins rich in arginine, and nucleoproteins rich in guanine, and also arises during autolysis of pancreas. The chief biological derivatives are:—

Monomethyl guanidine, $\text{CH}_3 \cdot \text{HN} \cdot \text{C}(\text{:NH}) \cdot \text{NH}_2$, traces of which occur in mammalian muscle (0.08 per cent.) and in urine. The urinary output is greatly increased after parathyroidectomy.

Glycocylamine, $\text{H}_2\text{N} \cdot \text{C}(\text{:NH}) \cdot \text{NH} \cdot \text{CH}_2 \cdot \text{COOH}$, guanidine acetic acid, is of interest as the precursor of creatine. It has been identified in the organism.

Creatine, $\text{H}_2\text{N}.\text{C}(\text{:NH}).\text{N}(\text{CH}_3).\text{CH}_2.\text{COOH}$, methylguanidino acetic acid, is the characteristic constituent of all vertebrate muscle. It is discussed subsequently along with its anhydride, creatinine.

Arginine, δ -guanidino valine, and canavanine are the only natural amino acids known to contain the guanidine group (p. 151).

Agmatine, δ -guanidino butylamine, $\text{H}_2\text{N}.\text{C}(\text{:NH}).\text{NH}.\text{(CH}_2)_3.\text{CH}_2.\text{NH}_2$, the amine obtained by decarboxylation of arginine, has been detected in ergot.

Galegine, an alkaloid found in Goat's Rue (*Galegia officinalis*), has been shown to be $\text{H}_2\text{N}.\text{C}(\text{:NH}).\text{NH}.\text{CH}_2.\text{CH}(\text{CH}_3)_2$.

Guanidine derivatives have a hypoglycæmic effect, and resemble insulin in causing a fall in the blood sugar level when injected into the circulation. The response, however, is due to a toxic interference with glucose production by the liver and does not represent a true sugar utilisation.

Analytical Reactions of the Guanidines.—(1) The *α -naphthol test* (Sakaguchi, 1925) is given by all compounds containing the grouping $\text{H}_2\text{N}.\text{C}(\text{NH}).\text{NH}.\text{R}$, and may be used to detect arginine, glyco-cyamine, methyl guanidine, agmatine and other substituted guanidines (p. 165). Free guanidine does not react.

(2) Free guanidine treated with 1:2-naphthoquinone-4-sodium sulphonate and alkali, yields after about ten minutes a brown solution which turns bright red on addition of nitric acid (Sullivan's test, 1935). Ammonia, methylamine and indole, but none of the natural guanidine derivatives, give similar reactions.

Normal blood has a "guanidine value" ranging from 0.0–0.2 mg. per 100 ml. This may be raised to 0.5 in conditions of arterial hypertension, nitrogen retention, eclampsia, and acute liver injury. It is possible, of course, that these guanidines, whatever they may be, are non-poisonous, and related to creatinine, which, itself, is retained in the blood in some forms of nephritis.

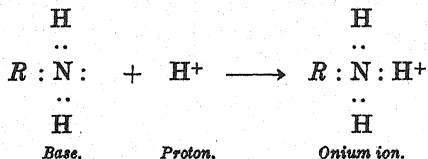
Adenosine Triphosphate, or ATP.

Severe shock, with fall in blood pressure, depression of renal function, and consequent uræmia, may follow extensive injury to muscle tissue due to crushing or deprivation of blood supply (ischæmia). The "muscle shock factor" has been identified by Green *et al.* (1943), and is adenosine triphosphate, the phosphate carrier in many forms of metabolism. On removal of all phosphate, it yields adenosine, which may be the actual shock factor in the general circulation.

The Onium Compounds

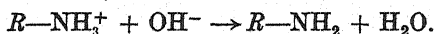
The substances previously considered owe their basicity to the presence of one or more amino groups in which the nitrogen atom is united by *covalencies* to two hydrogen atoms and another group,

thus completing an electron octet. In all these compounds, the nitrogen atom possesses a *lone-pair* of unshared electrons, by means of which it is able to form a *semi-polar* union with a proton ion or other positively charged ion. Biological amines are basic in the modern sense of being proton acceptors :



The term "onium" is applied to these cations in which nitrogen is exerting its maximum covalency, the most familiar example being NH_4^+ , the ammonium ion.

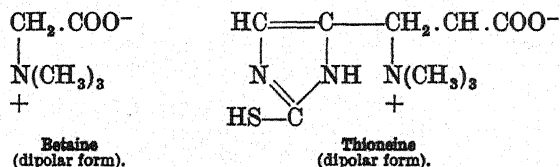
Cations of the type R.NH_3^+ , $\text{R}_2.\text{NH}_2^+$ and $\text{R}_3.\text{NH}^+$ are readily attacked by hydroxyl ions, which remove the proton to form water, and release the base,



However, when the onium ion is formed from four radicles, and is of the type R_4N^+ a compound results which is stable in aqueous solution and resembles the metallic cations in its power to form stable salts. The chief representatives of these onium compounds in biochemistry are the betaines, the cholines and amine oxides.

Betaines are cyclic bases found almost exclusively in plants, where they may be storage forms of nitrogen or detoxication products.

One member, ergothioneine, or thioneine, has been detected in mammalian blood. It is derived from thiol-histidine, an amino acid, unknown in natural sources.

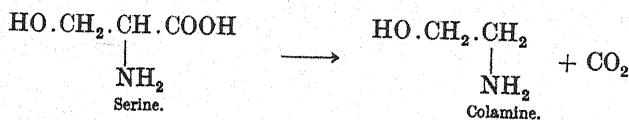


Both betaine and thioneine are neutral in solution owing to their dipolar structure (p. 58).

Refined beet sugar contains traces of betaine as a constant impurity, and thus may be distinguished from cane sugar.

Cholines, or Trimethylamine Bases.—This subgroup includes the highly active and widely distributed base *choline*, together with its derivatives, all of which may be regarded as offspring of the parent, β -amino-ethyl alcohol, or *colamine*, $\text{HO}.\text{CH}_2.\text{CH}_2.\text{NH}_2$.

Colamine is of interest as being a constituent of the cephalins (p. 181), and a precursor of the cholines. Its natural origin is unknown, the simplest source being decarboxylation of the hydroxy-amino acid, serine.



Choline, hydroxyethyl - trimethyl - ammonium hydroxide, was originally obtained from bile, and is widely distributed elsewhere in plants and animals as a constituent of lecithin. The choline content of mammalian tissue ranges from about 10 to 30 mg. per 100 gm. fresh material.

Properties of Choline.—Choline and its derivatives constitute, along with histamine, the chief *depressor bases* found in extracts of animal tissues. Intravenous injection of such extracts evokes a fall in the blood pressure of higher animals, the response often being variable and paradoxical. This is ascribed to: (i.) unidentified constituents, (ii.) synergic or reinforcement action among the bases, (iii.) species and other differences among the experimental animals, (iv.) interference by anaesthetics. Pure choline has a vagomimetic effect shown by its action in slowing the heart beat and stimulating the movements of the alimentary tract. In addition, it is a secretagogue, stimulating the salivary, sudorific, and lachrymal glands; a myotic, contracting the pupil of the eye; an ecboic, stimulating the isolated uterus to contract; and a lipotropic factor in nutrition.

Atropine inhibits the vagomimetic effect of choline and unmasks various secondary effects, notably a vaso-constriction. Hence, choline injected subsequently to atropine may exert a pressor action.

Acetyl choline attracted attention in 1909, when it was found to have an action similar to choline but 100,000 times more powerful. It is a natural constituent in extracts of ergot, and was subsequently identified in higher animal tissues, when Dale and Dudley (1929) obtained it from fresh spleen. Acetyl choline is the most powerful depressor base known. Intravenous injection causes a sharp and transient fall in blood pressure, due to arteriolar dilatation. If this effect be inhibited by atropine, it is seen that acetyl choline also stimulates ganglion cells in a way similar to nicotine, which, however, eventually paralyses the synapse.

Two other properties are of use in analysis. (1) In common with choline, muscarine, and other "parasympathetic" stimulants, acetyl choline increases the tone and rhythm of isolated rabbit intestine

suspended in warm saline. (2) Acetyl choline causes a characteristic slow wave of contraction in denervated mammalian voluntary muscle. This action is peculiar to the choline esters.

Acetyl choline is very unstable, and is hydrolysed when warmed with dilute alkalis. By this means it may be distinguished from the relatively more stable choline and histamine. Furthermore, it is rapidly destroyed by disintegrated splenic tissue, and its detection only possible in extracts prepared under special conditions.

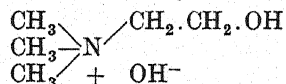
Choline nitrite, or pseudo-muscarine, was at one time thought to be identical with natural muscarine, the highly poisonous alkaloid of the mushroom *Amanita muscaria* (Fly Agaric), and to be the aldehyde corresponding to choline. Both suppositions are wrong. The natural alkaloid is much more toxic than choline nitrite, and its effects are antagonised by atropine, which has little protective effect against the synthetic ester.

Neurine is obtained during the putrefaction of choline or compounds containing choline, such as lecithin. It is a possible contaminant in all crude choline preparations, and a typical example of the class of bases formed during the putrefaction of tissues, and at one time called "ptomaines."

Neurine resembles choline in many physiological properties, but is much more toxic.

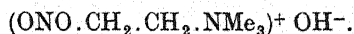
Structure of the Cholines.—Choline is a viscid alkaline liquid, stable in dilute aqueous solutions, but decomposed by heat into trimethylamine, $(\text{CH}_3)_3\text{N}$, and ethylene glycol, $\text{HO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{OH}$. It and its relatives are hydroxides of various onium compounds.

- (1) **Choline**, trimethyl-colamine hydroxide,



- (2) **Acetyl choline**, $(\text{CH}_3\text{COO} \cdot \text{CH}_2 \cdot \text{CH}_2 \cdot \text{NMe}_3)^+ \text{OH}^-$.

- (3) **Choline nitrite**, pseudo-muscarine,



- (4) **Neurine**, trimethyl-vinyl-ammonium hydroxide,



Physiological Properties of the Onium Bases.—Onium bases, in general, have three distinct physiological properties : (i.) curare-like paralysis of motor nerve endings in voluntary muscles ; (ii.) parasympathetic or "cholinergic" effect in stimulating tissues supplied by the parasympathetic system ; (iii.) nicotine-like action in paralysing sympathetic nerve ganglia. The intensity and the quality and duration of the particular effect depend both on the

structure of the choline derivative and on the previous history of the tissue attacked. Neither the betaines nor trimethylamine oxide have these properties, which must reside in the positively charged onium ions.

The chief significance ascribed to choline in nerve metabolism is its presence as the parent of acetyl choline, the characteristic neurocrine or effector substance of the parasympathetic nervous system. It also influences fat storage (p. 369).

NEURO-HUMORAL MECHANISMS

The existence of a neuro-humoral mechanism or chemical agent intervening between nerve impulse and tissue response has been demonstrated chiefly by the work of Loewi (1921-1930) on the heart, Sherrington on the reflex-arc, and Lewis on the cutaneous capillaries. These chemical agents, or *neurocrines* as they may be termed, resemble the autacoids (Chapter 24), but the principal effect is local and more or less limited to the site of their origin. At the same time, two of the best known neurocrines, namely, acetyl choline and adrenaline, function as typical hormones.

History.—In 1921, Loewi reported that the perfusion fluid from a frog's heart that had been stopped by vagal stimulation was able to induce typical vagal effects when perfused through another heart, from which he concluded that a "vagal substance" was liberated locally. The work was confirmed by other investigators, who showed that similar substances were liberated in various tissues and glands on stimulation of the parasympathetic nerves, and the term *parasympathin* was introduced to denote the reactant. The term *sympathin* was applied to the corresponding substance liberated locally on stimulation of the sympathetic nerves. Parasympathin is dialysable and acid-stable, but rapidly destroyed by alkalies and by esterases present in blood and most tissues. The destruction by esterases can be prevented by *eserine* (physostigmine), an alkaloid of the Calabar bean.

In all these properties, parasympathin resembles acetyl choline.

In many ways sympathin resembles adrenaline, the secretion of the suprarenal medulla. Following a suggestion of Dale, nerve fibres are now classified as *cholinergic* and *adrenergic*, according to the type of neurocrine liberated. The hormones are secretions of the nerve terminals, themselves, and not products of the excited tissue (Parker, 1932).

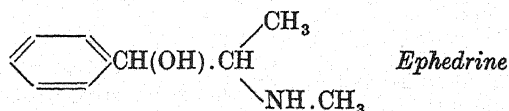
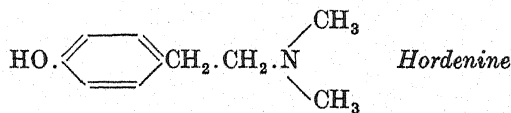
The manner in which a neurocrine operates is uncertain. Presumably it is set free by an activation or change in valency electrons in the parent substance as the result of the arrival of a nerve impulse.

The liberated neurocrine then reacts with some tissue constituent and starts the series of chemical changes involved in muscle contraction or gland secretion.

Neurocrines are characterised by (a) lability, and (b) sensitivity to specific antagonists. Lability is shown by the rapid disappearance of the free neurocrine, otherwise its effect would persist long after the cessation of the evoking stimulus.

Definition and Classification.—A neurocrine, or neuro-humoral factor, is a specific reactant liberated at a nerve ending as the result of nerve stimulation.

(1) *Sympathins*, the sympathomimetic substances liberated in the heart, intestine, and elsewhere as the result of adrenergic stimulation; one of them has been identified with adrenaline. Natural compounds with a sympathin effect are: vaso-pressin (p. 502), tyramine, *l*-adrenaline, hordenine (an alkaloid from barley) and *l*-ephedrine (an alkaloid from the Chinese plant, *Ephedra equisetina*), which acts by protecting adrenaline from destruction by a local oxidase.



(2) *Parasympathin*, the vagomimetic substance liberated in the heart and elsewhere as the result of stimulation of the vagus or an other cholinergic nerve supply. It is a choline ester, and has been identified with acetyl choline. The parasympathetic stimulant and powerful vaso-dilator, acetylcholine stimulates peristalsis, and decreases the heart rate. Subcutaneous or intramuscular injections of 50–100 mg. of acetyl choline chloride are used in treatment of vascular disorders, including gangrene, arteriole spasm, and Raynaud's disease. The drug is so potent that, to ensure rapid dilution by tissue fluids, it must not be administered by intravenous injection.

Stimulation of parasympathetic nerves or motor nerves to skeletal muscles releases momentarily at the nerve endings a little acetyl choline, which conveys the impulse across the myoneural junction. The effect is controlled by the rapid hydrolysis of the acetyl choline by choline esterase. If the esterase be inhibited by *eserine*, or the synthetic analogue, *prostigmine*, the effect of the

acetyl choline is prolonged. Eserine and prostigmine are used in treatment of myasthenia, intestinal paresis, and other muscular dystrophies.

Guanidine and its simple derivatives greatly sensitise muscle to acetyl choline, though they do not inhibit choline esterase.

The diffusion, but not the release, of acetyl choline at parasympathetic endings is prevented by *atropine*, an alkaloid from belladonna, which in consequence, inhibits parasympathetic stimulation response.

Curare, unlike atropine, prevents released acetyl choline from acting in the myoneural junction of skeletal muscle, but does not affect parasympathetic endings. Perfusates from heart, striated muscle and ganglion cells contain no acetyl choline, unless the tissues are stimulated, when it is obtainable from all three, although physostigmine may have to be used to protect it from choline esterase. In all three tissues, perfusion with acetyl choline induces the typical effects of nerve impulses : inhibition of heart beat, contraction of skeletal muscle, and discharge of ganglion cells. Conversely, the effect of acetyl choline can be checked, without affecting its liberation, by atropine (heart), curare (striated muscle), and nicotine (ganglion cells). Pilocarpine, the alkaloid from jaborandi, is a typical parasympathin, and its inhibitory effect on the heart is antagonised by atropine.

(3) *Synapsin*, the synaptic substance liberated at the synapses of the reflex-arc, determines the phenomena of "recruitment" and "after-discharge" displayed by the nervous system (Sherrington), and has been identified as acetyl choline.

(4) *Vaso-dilatin*, or "H-substance" (Lewis), is liberated locally as a result of injury to the skin, such as scalding, burning, irradiation and freezing. By direct action it is responsible for capillary dilation and wheal formation, and through an axon-reflex it evokes a red flush, or "flare," due to arteriolar dilation in the surrounding skin area. H-substance has been identified with histamine.

(5) *Angiotonin*, or hypertensin, is a heat-stable dialysable vaso-constrictor formed in the blood by the action of the renal enzyme *renin* on a plasma globulin.

Summary.—The principal vaso-pressor factors obtained from natural sources are : tyramine, tryptamine, ephedrine, adrenaline, pituitary vaso-pressin and angiotonin.

The principal depressor factors are : histamine, choline and its esters, adenosine, adenylic acid, and the less clearly defined substances, *angioxyl* (Kallikrein, or vagotonin), from pancreatic extracts ; and *carotidin*, from carotid gland.

CREATINE AND CREATININE

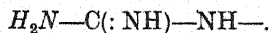
Creatine, methylguanidino acetic acid, or methyl glycocymamine, was discovered in meat extract by Chevreul, in 1835. Since then it has been shown to be a constant constituent of vertebrate muscle.

The adult human body has 90–130 gm. of creatine, 98 per cent. of which is in muscle, 1.5 in nerve, and 0.5 generally distributed. Entire blood contains 2–9 mg. per 100 ml., while plasma contains 0.5–3 mg.

Average values in mg. per 100 gm. fresh tissue are : man, 390 ; dog, 370 ; cat, 450 ; horse, 400 ; ox, 430 ; rabbit, 520 ; pigeon, 447 ; frog, 270–450 ; cod, 353 ; skate, 280. The total muscle creatine of a 70 kg. man ranges from 112–140 gm. Creatine is maximal in voluntary muscle, and minimal in involuntary muscle. Traces occur in other organs and tissues, usually one-twentieth or less of that found in the muscles of the same animal. Creatine appears to be absent from the muscles and other tissues of invertebrates.

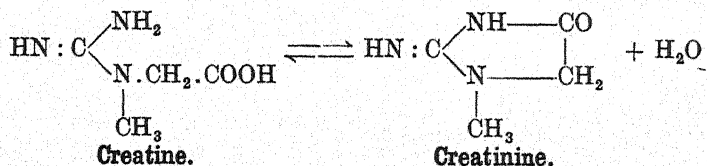
Properties and Reactions of Creatine.—Commercial creatine is now available as a by-product of the manufacture of meat extract, from which it can be purified by recrystallisation from hot water. It is obtained in hard, colourless prisms, soluble in water to the extent of 1 : 74 at 18° C., and freely soluble at 100° C. Unlike the natural bases, creatine is not precipitated by phosphotungstic acid.

Creatine gives a red colour when warmed in alkaline solution with dilute diacetyl (Harden-Walpole Test). Glycocymamine and arginine, free or in protein form, give a similar reaction, which is attributed to the presence of the guanidino group,



Creatine does not react typically with nitrous acid or with formaldehyde. On boiling with weak alkalis, it is hydrolysed to sarcosine and urea.

Dehydration.—On boiling with dilute acids, creatine may be converted completely into its anhydride *creatinine*. A similar change takes place slowly in aqueous solution, an equilibrium being reached, the constant of which depends on the temperature.



This conversion is the basis of the methods for estimating creatine.

Significance of Creatine.—Creatine is an essential reactant in the contraction process in vertebrate muscle. In the resting state, it is mostly combined with phosphoric acid as a non-diffusible *phosphagen*. Activity, fatigue, injury or death of the muscle causes the resolution of phosphagen into creatine and phosphoric acid, 150 calories being liberated for each gram of acid released. During the recovery stage, phosphagen is resynthesised, the energy for this being obtained by the breakdown of the carbohydrate in the muscle. Creatine thus serves as a phosphoric acid carrier in muscle metabolism.

Creatine phosphagen is only found in the muscles of vertebrates, its function among the invertebrates being fulfilled by arginine phosphagen (p. 355).

Creatinuria.—Creatine is a normal constituent of the urine of young vertebrates, and is almost always present in the urine of infants and children. It disappears from the urine of the adult man when the muscular system has reached the average percentage of total body weight. It persists intermittently in the urine of women, and is constant in the urine of many lower animals, including cattle, sheep, and the fox. Creatine is an important constituent of the urine of birds, being much in excess of the creatinine present. Creatinuria occurs even if the dietary be creatine-free, showing that it is a product of animal metabolism.

Types of Creatinuria.—(1) *Creatinuria of growth*, as found in children of both sexes up to puberty. It is ascribed to overproduction at an age when the storage capacity of the muscles is low, and is an overflow creatinuria.

(2) *Metabolic creatinuria* may follow nutritional disturbances due to excess of protein or deficiency of carbohydrate in the diet. It appears during pregnancy, lactation, hyperthyroidism, and diabetes.

(3) *Starvation creatinuria* is caused by autolysis of muscle during febrile conditions, starvation, and avitaminosis E.

Creatinuria is characteristic of the last months of pregnancy, and in the few days immediately preceding parturition it may be as high as 170 mg. *per diem*.

After parturition, the output rises to a maximum between the fourth and sixth day, and subsides to the normal value usually by the end of the month.

(4) *Creatinuria of low storage* is seen in muscular dystrophies and atrophies, where the administration of a small quantity of creatine leads to its rapid excretion in the urine.

CREATININE

Creatinine, or methylglycocymidine, the cyclic anhydride of creatine, was discovered in human urine by Heintz and by Pettenkofer, independently, in 1844, as a constituent precipitated by zinc sulphate or chloride.

Later investigation has shown creatinine to be a characteristic solute in all mammalian urine, ranking next to urea in quantitative importance.

Average percentages are : man, 0.06–0.2 ; goat, 0.038 ; ox, 0.11 ; sheep, 0.14 ; horse, 0.19. Creatinine also occurs in mammalian blood in concentrations of about 0.1–0.5 mg. per 100 ml. It is easily excreted by the kidney, being a non-threshold solute, and hence creatinine retention only occurs in severe renal dysfunction, when blood values greater than 4 mg. per 100 ml. may be reached. The creatinine content of tissues is about the same or less than that of the blood. Traces occur in many vegetable materials : cereals, potatoes, bran, straw, and soils.

Reactions of Creatinine.—(1) *Hydrolysis*.—On prolonged boiling in neutral or alkaline solution creatinine is in part slowly hydrolysed to creatine and in part resolved into urea and sarcosine. The transformation to creatine is complete if the creatine be removed from time to time by cooling and concentrating the solution.

(2) *Precipitation*.—Creatinine is more basic than creatine, and, unlike it, is precipitated by most of the alkaloidal reagents including phosphomolybdic and phosphotungstic acid. Warmed with Fehling's reagent in presence of sodium carbonate a slow-forming precipitate of creatinine cuprous oxide appears, and may mask a positive carbohydrate reaction in diabetic urine.

(3) *Analytical Reactions*.—These are considered in connection with the detection of creatinine in urine (p. 455).

Creatinine Excretion.—By the use of a colorimetric method, Folin, in 1904, showed that when the diet is free from creatine or creatinine the output of creatinine in the urine is "a constant quantity, different for different individuals, but wholly independent of quantitative change in the total amount of nitrogen eliminated."

Data such as these indicate that the creatinine output is independent of a tenfold increase or decrease in the nitrogen of the diet. Minor variations in output occur during long experimental periods, and are ascribed to variations in the body weight of the subject, especially as regards muscular tissue.

The Creatine-creatinine Metabolism of the Organism.—The creatine content of the human organism is maintained partly by the consumption of animal foodstuffs containing preformed creatine

Subject.	High Protein Intake, 10 gm. N. <i>per diem.</i>		Low Protein Intake, 1 gm. N. <i>per diem.</i>	
	Total Urinary N in gm.	Creatinine in gm. <i>per diem.</i>	Total Urinary N in gm.	Creatinine in gm. <i>per diem.</i>
1	12.2	1.58	4.1	1.48
	14.5	1.66	4.2	1.60
	16.1	1.49	3.8	1.57
2	11.5	1.63	5.3	1.58
	12.0	1.57	4.8	1.61
	14.7	1.58	3.6	1.60
3	14.6	1.05	2.7	1.17
	15.8	1.16	3.7	1.21
	14.4	1.12	2.8	1.13

and partly by the synthesis of creatine from identified precursors. In the absence of preformed creatine, the animal is able to meet all its requirements synthetically, as shown by the herbivora and other vegetarians. Human blood contains 2.9 mg. of creatine per 100 ml. Creatine, like other valuable metabolites, has a high renal threshold level. It is filtered from the plasma by the glomeruli, and reabsorbed during its passage along the tubule, probably by being phosphorylated by the renal cells, which thus produce a favourable diffusion gradient. When the creatine content of the blood exceeds 7 mg. per 100 ml. it exceeds the renal threshold level. Creatine enters the tubule more rapidly than it can be reabsorbed, and the excess escapes in the urine.

Creatinine, on the other hand, is a waste product, and of no use to the organism. It has a minimal renal threshold value, and though the blood creatinine content is assessed at 0.1–0.5 mg. per 100 ml., these figures probably include other compounds. Creatinine is filtered from the plasma by the glomeruli, but does not undergo reabsorption during its passage along the tubules. Its excretion rate is the most constant of any of the urinary solutes, being dependent primarily on the blood flow through the kidney. The creatinine of the plasma is one of the first solutes to increase in conditions of renal dysfunction involving nitrogen retention, and Myers claims that a blood creatinine value above 1.5 mg. per 100 ml. is early evidence of renal trauma.

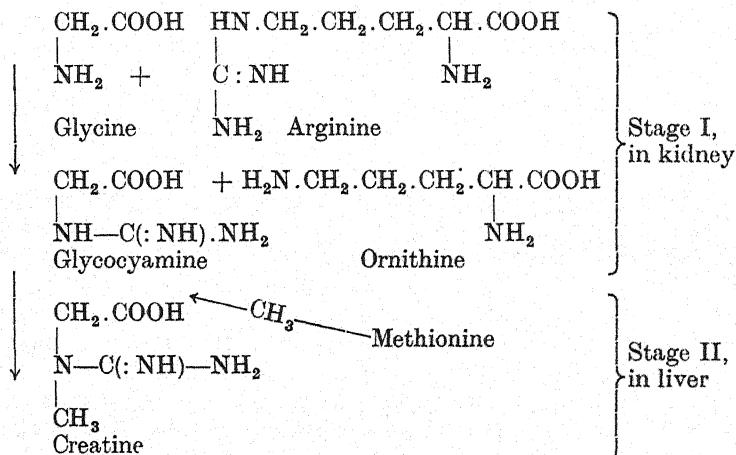
In the resting muscle, at least 95 per cent. of the creatine is in the bound form of creatine phosphagen, and is maintained at this level

by the plasma creatine, which is in equilibrium with the free creatine of the muscle. Creatine is continually being synthesised in accordance with the phosphagen requirements of the muscular and possibly other systems, the surplus being excreted as creatinine.

The Precursors of Creatine.—Tissue-slice experiments, using substrates labelled by isotopes, show that the creatine molecule is assembled in two stages :—

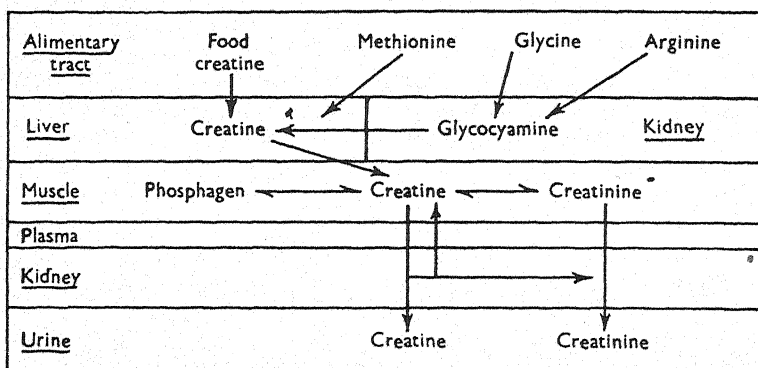
(1) Formation of glycoxyamine from glycine by transfer of the *amidine* group, —C(:NH)—NH_2 , from arginine. This “transamidination” occurs in kidney tissue, but not in liver. Both arginine and glycine have been long regarded as possible creatine precursors (Thompson, 1918 ; Brand, 1929), and may come from dietary proteins, or be synthesised in the animal.

(2) Formation of creatine from glycoxyamine by transfer of a methyl group from methionine. This transmethylation occurs in liver, but not in kidney tissue.



Creatine Construction (Borsook and Dubnoff, 1940).

The conversion of glycoxyamine into creatine has been demonstrated in the rat, by Bloch and Schoenheimer (1940), using ^{15}N as a tracer isotope. They later found that labelled creatine when given in the diet reappeared in the urine as creatinine, whereas no evidence was obtained that labelled creatinine in the diet can be converted into creatine. Wider aspects of the creatine-creatinine relationship are discussed by Beard (1943).



Creatine-Creatinine Metabolism

Miscellaneous Bases

Ammonia.—Although ammonia is formed by the deamination of amino acids and purines in many parts of the body, the free ion is found only in urine as a normal constituent. Traces of ammonia appear in saliva and in intestinal contents as a result of bacterial changes.

Circulating blood, as Conway has shown, has an ammonia value of zero, or below analytical level. After shedding, ammonia arises from three sources: (i.) α -ammonia, which appears almost immediately, and has a nitrogen value of 40% per 100 ml. It comes from the action of plasma deaminase on plasma adenosine, which, in the circulation, is protected by plasma CO_2 .

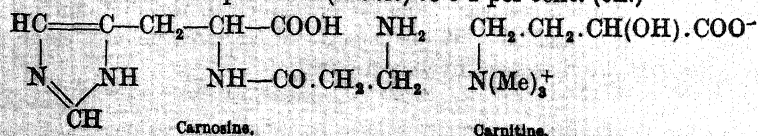
(ii.) β -Ammonia (1 mg. per 100 ml., in rabbit's blood), arises later, from deamination of adenosine triphosphate in the red cells.

(iii.) γ -Ammonia (0.35 mg. per 100 ml. in the rabbit) arises finally, from adenylic acid of the yeast-type.

Carnosine, β -alanyl histidine, forms about 0.2–0.3 per cent. of mammalian muscle. It is water-soluble, dextro-rotatory, and like histidine, gives a diazo reaction, and acts as a pressor base. Its significance is unknown but it is of interest in that it is a natural derivative of a β -amino acid.

Anserine, or methyl carnosine, occurs to the extent of about 0.1 per cent. in the muscles of the goose and other birds, where it appears to replace carnosine.

Carnitine is an onium base present in mammalian muscle in amounts from 0.02 per cent. (rabbit) to 0.1 per cent. (ox.)



Trimethylamine, $(\text{CH}_3)_3\text{N}$, occurs in the sexual tissues of many plants and animals, in association with the sex hormones.

Trimethylamine oxide, $(\text{CH}_3)_3\text{N}^+\text{O}^-$, an onium salt found in marine but never in fresh-water fishes. Rich sources are cephalopods and crustaceans, the muscle of the lobster containing about 0.3 per cent. The oxide is soluble, non-toxic and almost neutral, and is an important excretory form of nitrogen. Among the elasmobranchs it serves in the maintenance of fluid equilibrium, and is responsible for 20–25 per cent. of the total osmotic pressure of the blood. It is rapidly decomposed by post-mortem autolytic and bacterial changes, and the liberated trimethylamine, $(\text{CH}_3)_3\text{N}$, characterises the odour and taste of stale marine fish.

Tetramethyl ammonium hydroxide, $(\text{CH}_3)_4\text{N}^+\text{OH}^-$, is the toxin in the stings of jellyfish, and displays marked curare-like properties.

Nicotinic amide, $\text{C}_5\text{H}_4\text{N}.\text{CO}.\text{NH}_2$, is a constituent of the co-enzymes I and II, and as such acts as a hydrogen carrier in tissue respiration. The amide is derived from nicotinic acid, or carboxy pyridine, and is combined with ribose phosphate and adenosine in nucleotide structure in the co-dehydrases. Its hydrogen-carrying power is due to the reducibility of the onium nitrogen in the pyridine ring (p. 381).

Benzedrine, $(\text{C}_6\text{H}_5)\text{CH}_2.\text{CH}(\text{NH}_2).\text{CH}_3$, β -phenyl isopropylamine, a synthetic base, was introduced into therapeutics in 1935. It is an adrenergic type of base, resembling ephedrine. Both these amines inhibit the amine oxidase that destroys adrenaline, and for this reason both prolong the effect of sympathetic stimulation. Quastel and Wheatley (1933) have shown that aromatic amines, such as β -phenyl ethylamine, tyramine and indole, by producing aldehydes, inhibit strongly the oxidation of glucose, lactate and pyruvate by brain tissue. Benzedrine (amphetamine) and related compounds, by repressing aldehyde production, act as mental stimulants and prolong wakefulness.

PLANT ALKALOIDS

Characteristic of many species of plants are the presence of complex derivatives of pyridine, quinoline and other heterocyclic nuclei. Because of their basic properties, these substances are termed alkaloids. In complexity of structure and obscurity of function, the plant alkaloids excel the known animal bases, and they are the subject of specialised study because of the powerful and often unique physiological properties which many of them possess.

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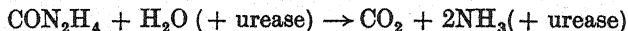
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CHAPTER 22

UREA

Urea, or "Carbamide," CON_2H_4 , is the principal form in which nitrogen leaves the higher organism, and is the chief nitrogenous constituent of the urine of vertebrates other than birds and snakes, where it is replaced by uric acid. Urea is present also in lower animals, in fungi, and in some higher plants. It is one of the simplest and most widely distributed of the biological nitrogen compounds.

History.—The ammoniacal fermentation of urine, which takes place spontaneously when urine is exposed for some days to the air, depends on the conversion of urea into ammonium carbonate by the urease-forming micro-organisms that abound in the environment.



This change had been known before the time of Pliny, and, in 1773, Rouelle isolated urea by alcoholic extraction of the residue got by the careful evaporation of fresh urine. In 1798, Fourcroy and Vauquelin obtained urea nitrate, $\text{CON}_2\text{H}_4 \cdot \text{HNO}_3$, as a crystalline precipitate on addition of excess of concentrated nitric acid to the urine of man and other animals, and demonstrated that "this special material of the urine, which we now call urea [Urée], . . . gives rise to the carbonate of ammonia which replaces it in the putrefaction of urine."

Wöhler, in 1828, announced his famous synthesis of urea from inorganic materials, and, in so doing, bridged the abyss between organic and mineral chemistry.

The synthesis was the result of an attempt to prepare ammonium cyanate by the action of ammonium chloride on silver cyanate, or ammonium hydroxide on lead cyanate. In each reaction a crystalline product was obtained which was identical with urea, previously found only in urine. The mechanism of the synthesis has been explained by Werner. Cyanic acid, HOCN , readily changes into its isomer, *iso*- or keto-cyanic acid, $\text{HN}:\text{CO}$, which unites with ammonia directly to form urea.



Distribution of Urea in Animals.—Urea is present in the blood and tissue fluids of all vertebrates and in the urine of all mammals. It also occurs in many invertebrates, including Echinoderms,

Crustaceans, Molluscs, and Nematodes, the usual concentration range being 16-70 mg. per 100 ml.

Elasmobranchs (Dog fish, Skate, Shark) are noteworthy for having an exceptionally high concentration of blood urea, which may reach 1.7 per cent.

As a solute, urea is universally and uniformly distributed throughout the organism, the concentrations being approximately the same as in the blood. Outstanding exceptions are the adipose tissue, which has a low urea value corresponding to its low content of water, and the renal tissue, which has a high value, owing to the presence of urine.

Excretion of Urea.—Urea leaves the animal in three ways: the kidney, the skin, and the intestine. Of these, the first is by far the most important, but in conditions of renal inefficiency the cutaneous excretion of urea may become considerably greater than its normal human value of 0.1 gm. *per diem*. Little is known about the intestinal excretion of urea; in health it is probably negligible.

The urinary output of urea depends primarily on the protein content of the dietary. On a European standard of 80-100 gm. protein *per diem*, the daily output is about 28 gm. urea, representing 80-89 per cent. of the total urinary nitrogen.

According to Van Slyke, when the rate of secretion of urine exceeds an "augmentation limit" of 2 ml. per minute, its urea content is directly proportional to the urea in a given volume of blood passing through the kidney in unit time, the maximal value of which is about 75 ml. of blood per minute, which affords the "maximum clearance" of urea from the blood.

The urea content of mammalian urine is greatest in Carnivora, average percentage values being dependent on protein intake. *E.g.*, Man, America, 2.6; France, 2.2; Germany, 1.9; tiger, 6.9; cat, 2.2; rat, 4.5; cow, 0.9-2.6; horse, 1.5-2.6; rabbit, 0.2-0.4.

In human secretions other than urine, urea values, in mg. per 100 ml., are: blood, 15-40; cerebro-spinal fluid, 10-40; saliva, 10-35; milk, 16-30; bile, 30-80. Nitrogen values are sometimes expressed in terms of *urea nitrogen*, which is 28/60, or, approximately, half the value expressed as urea. Human blood has an average urea nitrogen content of 16 mg. per 100 ml.

Among plants, urea is found in many fungi and moulds in fairly high concentration. Green plants contain traces, probably as unstable ureides, or carbamido acids, such as citrulline.

Properties of Urea.—Urea is a white, crystalline solid, with a faint, salty taste. It separates from aqueous solutions in long prismatic crystals that melt at 132.6° C. (corr.), and above this

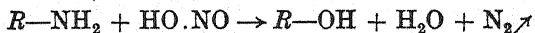
temperature dissociate into cyanic acid and ammonia. Urea is very soluble in water, glycerol, and methyl alcohol, and to a less extent in hot ethyl alcohol and in acetone. It is insoluble in ether, chloroform, and benzene.

Solubility in water, expressed in gm. urea per 100 gm. of solvent, is: 55.9 at 0° C.; 66.0 at 10° C.; 79.0 at 20° C.; 93.0 at 30° C.; 106.0 at 40° C.; 120.0 at 50° C.; 145.0 at 70° C. (Speyers, 1902).

Urea is neutral, but can react as a monobasic amide and form crystalline salts with strong acids. These, especially the nitrate and the oxalate, are insoluble in excess of acid, and may be used for the separation and identification of urea.

In alkaline solution, urea forms insoluble compounds with salts of the heavy metals, such as AgNO_3 and HgCl_2 .

Nitrous acid specifically attacks the group $-\text{NH}_2$ in aliphatic compounds, and replaces it by $-\text{OH}$, the nitrogen escaping as a gas:—



This reaction is employed by Van Slyke in a well-known method for estimating amino acids. The response of urea is altogether atypical. When nitrous acid, freshly prepared by the addition of a nitrite to a slight excess of acetic acid, is brought into contact with urea the mixture may remain unchanged for hours. If, however, a strong acid (hydrochloric or nitric) be added, a brisk reaction begins, and is completed rapidly, even in dilute solution, but the volume of nitrogen gas evolved is always less than the amount of urea decomposed. This suggests that the amide groups of urea are masked in neutral or faintly acid solution, and only partially exposed by the action of strong acids. Aldehydes react with urea only if the solution is sufficiently acid or alkaline to unmask the $-\text{NH}_2$ (p. 155).

Preparation of Urea from Urine.—(a) *As Urea Nitrate.*—When normal human urine is treated with an equal volume of concentrated nitric acid crystals of urea nitrate may begin to separate out within an hour. Many specimens of urine, however, yield no precipitate even after twenty-four hours. This is not always due, as might be expected, to a lower concentration of urea in the urine, but depends on the presence of a colloid that inhibits precipitation. An aqueous solution of urea of the same concentration as that in urine (2 per cent.) gives a dense precipitate of urea nitrate within ten minutes after addition of an equal volume of nitric acid, if the liquid be kept cool. Consequently, in preparing urea nitrate from its natural source, the urine must be concentrated to about a quarter of its original volume by evaporation on a water bath. An equal volume

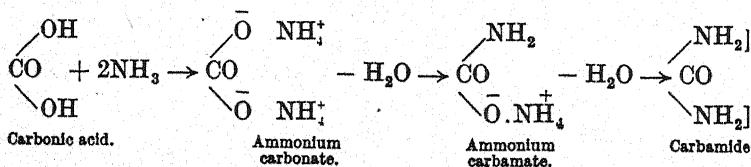
of pure nitric acid is added to the cold concentrate, and after twenty minutes the crystalline precipitate of urea nitrate is filtered off, neutralised with barium carbonate suspension, evaporated to dryness on a water bath, and the urea extracted by hot alcohol.

(b) *As Free Urea.*—The urine is evaporated to a paste on a water bath, and then extracted repeatedly with hot acetone. Urea thus obtained is very crude, and must be decolorised by being boiled with charcoal before it is purified by recrystallisation from acetone.

The Constitution of Urea.—Although urea is a simple compound built up of eight atoms, at least six different formulæ have been proposed to explain its constitution and reactions.

(1) *The molecular formula*, CON_2H_4 , follows from the fact that urea has a molecular weight of 60, as shown by the osmotic pressure and freezing point of the solution.

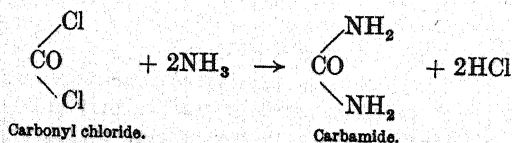
(2) *The carbamide formula*, $\text{CO}(\text{NH}_2)_2$ (Dumas, 1830). According to this early formula, urea is the diamide of carbonic acid and can be prepared by the general reaction for the preparation of amides, namely, the dehydration of the corresponding ammonium salt.



Ammonium carbamate is obtained by heating ammonium carbonate, and the further dehydration to carbamide seems obvious.

This synthesis was first accomplished by Basarov in 1868, who obtained a 3 per cent. yield of urea after heating ammonium carbamate for four hours under pressure at 140°C .

The synthesis of urea from the action of ammonia on carbonyl chloride appears to proceed in accordance with the carbamide formula :—



The carbamide formula for urea is widely accepted, and serves to explain, superficially at least, many of the reactions of the compound.

(3) *The amidine or iso-carbamide formula*, $\text{HO} \cdot \text{C}(\text{NH}) \cdot \text{NH}_2$

(Butlerov, 1868).—Urea is a neutral solute, and has not the alkalinity implied by the presence of two amide groups. With strong acids only monobasic salts are formed, for example, urea nitrate, $\text{CON}_2\text{H}_4 \cdot \text{HNO}_3$. The difficulty with which urea is obtained by the action of heat on ammonium carbamate is in striking contrast with the ease with which amides are prepared from the corresponding ammonium salts.

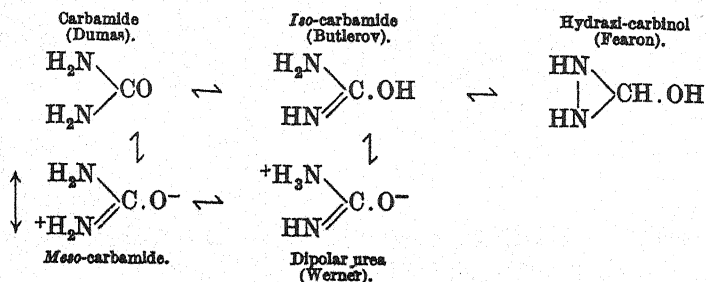
These and other considerations led Butlerov to infer that urea existed in solution as an isomer of carbamide, and he appears to have been the first to suggest the formula, subsequently adopted by Wanklyn and Gamgee (1868), according to which urea is an unsymmetrical structure possessing only one amido group.

The dipolar formula, $-\text{O} \cdot \text{C}(\text{NH}) \cdot \text{NH}_3^+$ (Werner, 1912–1923).—To explain the absence of typical amide properties, and to account for the readiness with which urea is formed from, and dissociated into, ammonia and cyanic acid, Werner, in 1912, proposed a formula for urea in which the amide and hydroxy groups were held by mutual attraction in neutral solution and released by the presence of strong acids or alkalis. When the formula was first proposed the existence of dipolar or zwitter-ions had not been accepted by chemists, and Werner represented the urea as a cyclic structure. This has been criticised on the grounds that one of the nitrogen atoms is depicted as pentavalent, which was not Werner's intention. The dipolar form reverts to the amidine form under the influence of acids, alkalis or heat, in solution, although there is an uncertainty as to whether it is the amido group or the imino group which carries the positive charge (Sidgwick, 1936). That is to say, crystalline urea may be $-\text{O} \cdot \text{C}(\text{NH}) \cdot \text{NH}_3^+$ or $-\text{O} \cdot \text{C}(\text{NH}_2)\text{NH}_2^+$.

(5) *The Cyclic Formula*.—Urea is a neutral solute, and has neither the base-neutralising power implied by the presence of an $-\text{NH}_3^+$ group, nor the acid-neutralising power implied by $-\text{NH}_2$. Unlike the amino acids, which are typical dipolar compounds, free urea does not immediately react with formaldehyde nor with nitrous acid, but only does so when in strongly acid solution. This suggests that the amide groups are masked by mutual combination, possibly as a *cyclic amidine* or *hydrazine* ring that requires to be unlocked by a strong acid or alkali before the groups can react.

(6) *The resonance formula, meso-carbamide*.—Measurements of the physical properties of urea, such as the Raman spectrum, the dipole-moment and the parachor, yield data, some of which are not yet fully interpreted, but which indicate that urea in solution is a mixture of isomers in a resonance equilibrium that is sufficiently stable to conceal the chemical properties of the amido group. Urea is thus represented as a hybrid structure, *meso-carbamide*, repre-

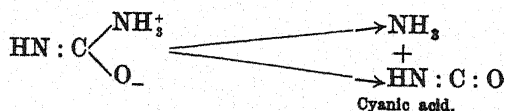
senting a mixture of the dipolar forms (Pauling, *et al.*, 1935 ; Bell, *et al.*, 1943).



Five Structural Formulæ for Urea

REACTIONS OF UREA (Werner, 1923)

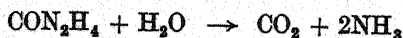
(1) **Decomposition by Heat.**—Heated above its melting point (132° C.) urea readily dissociates into cyanic acid and ammonia :—



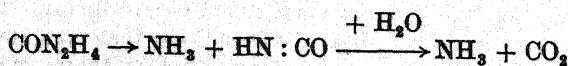
Cyanic acid is highly reactive and combines with the residual urea to form biuret, $\text{H}_2\text{N.CO.NH.CO.NH}_2$, which gives a rose colour with copper sulphate and an alkali.

(2) **Hydrolysis.**—Urea in sterile solutions is stable at ordinary temperatures, but if boiled alone, or in presence of alkalis or acids, it is converted eventually into ammonia and carbon dioxide.

The change, usually described as the hydrolysis of urea, is represented by the equation :—



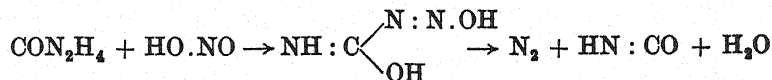
According to Werner, what happens is first the dissociation of urea into ammonia and cyanic acid, and then the hydrolysis of cyanic acid to ammonia and carbon dioxide.



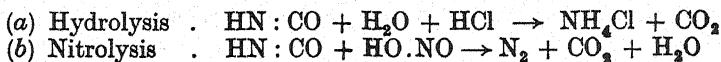
Urea solutions kept under ordinary laboratory conditions soon become alkaline owing to the action of micro-organisms. Until this

was recognised it was accepted as an example of the spontaneous reversion of urea into ammonium cyanate.

(3) **Deamination by Nitrous Acid.**—The condition necessary for attack is the addition of a sufficiently strong acid (*e.g.*, HCl) to unmask the amide group, which undergoes (*a*) diazotisation, followed by (*b*) decomposition into nitrogen and cyanic acid.

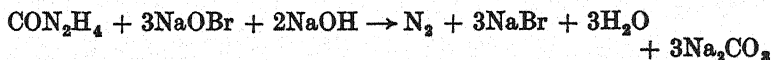


As it is generated, cyanic acid is decomposed by two independent reactions :—



The fixation of some of the cyanate nitrogen as ammonia and as a nitrate accounts for the yield of gaseous nitrogen invariably being less than the theoretical amount.

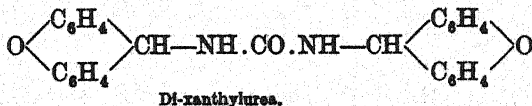
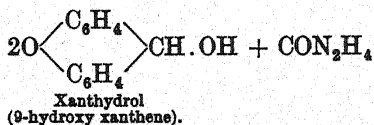
(4) **Deamination by Hypobromite.**—This reaction underlies the gasometric method of estimating urea. The condition necessary for attack is brought about by the addition of an alkali, which unmasks the amide groups. Subsequent addition of hypobromite leads to bromination of the amide groups, followed by simultaneous hydrolysis and oxidation.



Owing to secondary reactions this change is never quantitative.

The chief secondary reactions are : (*a*) formation of cyanate and hydrazine ; (*b*) formation of carbon monoxide. These three compounds have been detected by independent workers. The conditions governing their formation have been investigated by Werner.

(5) **Condensation with Xanthidrol.**—Xanthidrol condenses with urea in acid solutions to form an extremely insoluble di-xanthylurea :



Owing to its low solubility, the xanthydrol is used as a 10 per cent. solution in methyl alcohol, and the urea solution must contain a large excess of glacial acid to keep the free xanthydrol from being precipitated by water, and also to unmask the amido groups so that they will react. The test will reveal urea in concentrations of 1 : 10,000 in about fifteen seconds; and 1 : 800,000 in ten minutes. A positive result is shown by a crystalline precipitate of di-xanthylurea slowly appearing as a cloud of fine, colourless, silky needles.

It is important to note that xanthydrol is unstable both in solid form and in solution, and soon loses its precipitating power. It should be prepared as required by the reduction of xanthone.

The reaction is not given by any of the natural amino acids, purines, pyrimidines, or simpler natural bases, and hence is of great biochemical value. Monosubstituted ureas, thiourea, urethane, and biuret, however, yield sparingly soluble condensation products.

The practical details of the technique are described by Fosse and by Werner.

(6) **Decomposition by Urease.**—Urea is rapidly and completely converted into ammonia and carbon dioxide by the widely distributed enzyme urease which is found in the seeds of many leguminous plants and in many micro-organisms. The chief sources of urease are the seeds of the Jack Bean (*Canavalia ensiformis*), the Sword Bean (*C. gladiata*), and the Soy Bean (*Glycine hispida*). The optimal region of the reaction is about pH 7, and the change follows the simple equation :



The zymolytic decomposition of urea differs from the hydrolysis in that carbamic acid is the first identifiable product of the reaction (Sumner, 1926).

(7) **Analytical Reactions of Urea.**—(a) *Biuret Test.*—Solid urea is heated above its melting point for a few minutes. Fumes of ammonia are evolved, and a white deposit of ammonium cyanate condenses on the sides of the tube. After cooling, the residue is dissolved in water, and treated with sodium hydroxide and a few drops of dilute copper sulphate. A rose-pink colour shows the presence of biuret, which has been formed by union of the liberated cyanic acid with unchanged urea (p. 164).

(b) *Benzylidene Test.*—The solution is acidified with a few drops of HCl, and then a few drops of Ehrlich's aldehyde reagent (3 per cent. *p*-dimethylaminobenzaldehyde in 20 per cent HCl) are added. An intense yellow colour develops if urea be present.

The colour is due to the formation of *p*-dimethylaminobenzylidene urea. Similar colours are given with compounds containing the —NH—CO—NH₂ grouping, such as biuret. Alkalies and excess of strong acid discharge the colour. Nitrites interfere by forming deep yellow nitro derivatives that are not bleached by alkalies.

(c) *Hypobromite Test*.—When alkaline sodium hypobromite is added to urea a vigorous effervescence occurs with liberation of free nitrogen. This reaction is widely used in the estimation of urea in urine, but its delicacy is limited by the fact that all the urea nitrogen is not evolved as gas, and also many other biological compounds, such as ammonia and amino acids, evolve nitrogen.

(d) *Urease Test*.—This is the most selective test for urea. The neutral solution is incubated with a urease preparation and the liberated ammonia is detected by means of indicators, and estimated by titration or by colorimetry.

(e) *Xanthidrol Test*, which has been adapted for analytical use by Fosse (1928).

(f) *Diacetyl Test*.—A few drops of the urea solution are mixed with excess (2–3 ml.) of concentrated hydrochloric acid and 2–3 drops of 3 per cent. diacetyl monoxime. On boiling, a yellow colour develops if urea be present. The colour changes to deep orange on careful addition of an oxidiser (1–3 drops of 0.1 per cent. hydrogen peroxide or 1 per cent. potassium persulphate). Substituted ureas, including citrulline, give red pigments (p. 166). The test is delicate and will reveal 0.1 mg. of urea. It has been adapted for blood urea estimations by Ormsby (1942), and by Kawerau (1946).

The Origin of Urea in the Animal Body : Ureagenesis

Ammonia is a characteristic end-product of amino acid metabolism. It is very poisonous, and must be detoxicated prior to excretion. In mammals, and many other types, this is done by conversion to urea, and excretion as a urinary solute. In birds and reptiles, ammonia is converted into the sparingly soluble uric acid.

The adult human body, on an ordinary daily diet containing 80–100 gm of protein, manufactures and excretes upwards of 25 gm. of urea, *per diem*, which represents more than 11 gm. of ammonia. Consequently, *ureagenesis* is a process of the greatest importance. The mechanism is highly efficient, and there are no pathological records of its complete failure in any known disease.

Location of Ureagenesis.—It has been suggested that ureagenesis is a property of most tissues, but perfusion and incubation experiments have shown that the mechanism is narrowly restricted to the liver, and there is no evidence that urea can be assembled from ammonia and carbon dioxide in any other tissue in the mammal, although the presence of arginase in the kidney may account for a slight subsidiary extra-hepatic production of urea from surplus arginine.

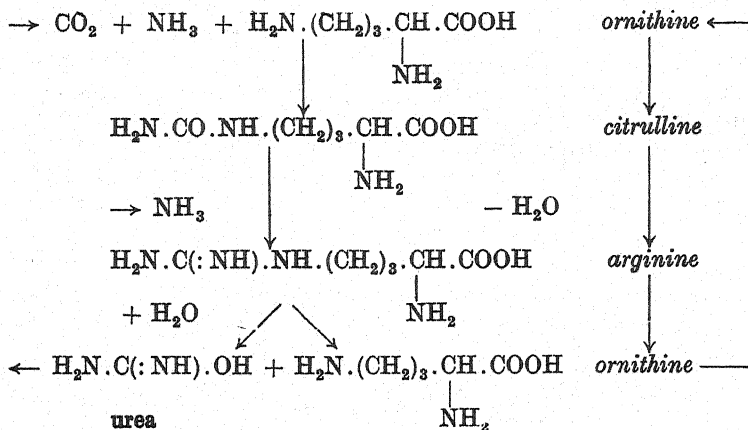
The unique significance of the liver was demonstrated experi-

mentally in 1927 by Mann and his colleagues, who showed that total hepatectomy in dogs resulted in complete cessation of ureagenesis. The amino nitrogen and the ammonia of the blood, urine and tissues increased, while, at the same time, the urea of the blood and tissues decreased, owing to excretion in the urine. If the kidneys are ligatured, the blood urea remains constant in the hepatectomised animal.

Mechanism of Hepatic Ureagenesis. (1) *The Ornithine Cycle of Krebs and Henseleit* (1932). Although urease can produce urea from ammonia and carbonic acid by a reversion synthesis under suitable conditions, urease is absent from mammalian tissues, apart from traces found in gastric mucosa, and therefore cannot be concerned in ureagenesis. Arginase is the only enzyme known to liberate urea; and arginine was, until the recent discovery of canavanine, the only amino acid known to form urea on simple hydrolysis. In 1914, Clementi pointed out that arginase is present in the livers of all animals that excrete nitrogen as urea, but is absent from animals, such as birds and reptiles, that are *uricotelic* in that they excrete nitrogen as uric acid.

This suggested that arginase has a function in metabolism more important than the mere removal of surplus dietary arginine. The missing component in the system was provided by the discovery of citrulline, and the proof, in 1930, that it was a uramido acid midway in structure between ornithine and arginine. By employing a tissue-slice technique that enabled them to work with intact and actively respiring tissues, Krebs and Henseleit were able to show that the liver is the only organ capable of synthesising urea from ammonium carbonate, and, furthermore, that the rate of the synthesis was greatly increased by addition of one of the three amino acids, ornithine, citrulline or arginine. No increase in the rate of ureagenesis was observed on addition of any of the other natural amino acids or related compounds. In this process, the ornithine appeared to act as a catalyst or carrier, since it is not used up, and small amounts are able to bring about the synthesis of an indefinite quantity of urea, provided that ammonia and carbonic acid are continuously supplied.

From these facts, Krebs has explained urea formation in the animal body in terms of an ornithine cycle made up of three stages: (i.) Formation of *citrulline* by condensation of one molecule of ammonia and one of carbon dioxide with the δ -amino group of ornithine, (ii.) formation of *arginine* by condensation of a second molecule of ammonia with the citrulline, (iii.) decomposition of arginine by arginase, with formation of *urea* and *ornithine*, which rejoins the cycle.



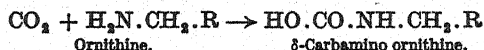
ornithine \leftarrow
 ↓
 citrulline
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 arginine
 ↓
 ornithine

The Ornithine Cycle

The maximum yield of urea from the rat liver is about 4 per cent. of the dry tissue weight per hour, under normal conditions, but can be increased three-fold by addition of citrulline. Urea synthesis is not necessarily accompanied by oxygen uptake, but can proceed anaerobically in presence of glucose, lactate or pyruvate as sources of energy.

At least three catalysts are required in the ornithine cycle, and of them, only one, namely *arginase*, has been separated, and will work in solutions; the others require the presence of intact liver tissue capable of respiration.

The obscure stages in the cycle are (i.) the conversion of ornithine into citrulline, and (ii.) the conversion of citrulline into arginine. According to Krebs (1936), the first of these depends on the combination between CO_2 and the δ -amino group of citrulline to form a carbamino compound,

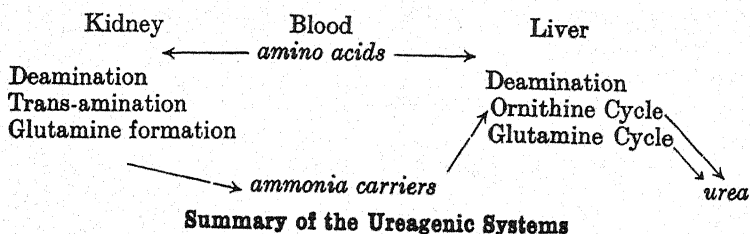


The carbamino compound is then combined with ammonia, and dehydrated to form the uramido acid, citrulline, $\text{H}_2\text{N} \cdot \text{CO} \cdot \text{NH} \cdot \text{R}$.

Using isotope-labelled CO_2 , it has been found that the carbon in urea is derived from the H_2CO_3 of the tissue fluids (Evans, *et al.*, Schoenheimer, *et al.*, 1940), thus proving that the ureagenic cycle is fed by CO_2 . Gornall (1942) reports that citrulline accumulates in liver slices suspended in saline containing ornithine, CO_2 , NH_4^+ , and lactate. Regardless of the nature of the mechanism, the synthesis of urea from NH_3 and CO_2 , under physiological conditions, entails an energy gain of nearly 14 kilocal. per gm. molecule of urea formed, so the cycle must be coupled with some system supplying energy.

Objections to the ornithine cycle as the exclusive or chief source of urea have been raised by Bach (1939-42), Trowell (1941), and others (Borsook and Dubnoff, 1943). The observation that citrulline is rapidly converted to arginine by kidney but not by liver tissue, suggests a possible distribution of the cycle between these organs.

(2) *The Glutamine Cycle of Leuthardt* (1938).—In presence of liver tissue of cat, guinea-pig, or rat, glutamine produces 40–60 per cent. more urea than what would be obtained from an equivalent amount of ammonia. Unlike the ornithine cycle, the process is not dependent on tissue respiration, and free ammonia is not utilised. Leuthardt, the discoverer of the reaction, believes that it represents an independent and important mode of ureagenesis. The glutamine reacts with carbonic acid to form urea and pyrrolidine-2-carboxylic acid, which undergoes successive transformation to proline and glutamic acid, and subsequent amination to glutamine, thus completing the reaction cycle. Asparagine resembles glutamine in ureagenic properties.



Significance of Urea in the Animal Body.—(1) *A Detoxication Product.* The primary function of urea is to provide a stable, inert and very soluble form in which nitrogen can be eliminated, and thus removes the potentially toxic ammonia group from metabolic processes. The extent to which ammonia is converted into urea depends on the base-balance of the organism. In conditions of acidosis, ammonia may be diverted from ureagenesis, and used to depress the H-ion concentration of the tissues by combining with protons. This is shown by the fact that the excretion of ammonium ions in the urine is increased by acidogenic dietaries.

(2) *A Diuretic.*—During its renal excretion, urea carries with it sufficient water to keep its concentration below 2·5 per cent., and thus acts as a natural diuretic. This property is the basis of several tests for renal efficiency.

(3) *A Uræmic Toxin.*—Urea was formerly believed to be responsible for the pathological condition of *uræmia*, which is due to defective renal excretion and consequent rise in blood urea. Urea, however, is not an obviously harmful compound, and, although very marked hyper-uræmia is found in some forms of nephritis, it is probable that the toxic signs and symptoms are due to a *uro-toxæmia* from retention of other urinary solutes, of which ammonium ions are the most dangerous. Sustained hyper-uræmia may, however, give rise to changes in tissue proteins, and toxic metabolites may, in theory,

be produced from urea. The opinion prevails that even if urea is not responsible for the uræmic syndrome, its concentration in the blood affords a good index of the severity of the disease.

The toxicity of large doses of urea is determined by the inability of the animal to excrete urea beyond a certain maximum rate which is about 1.5 gm. urea per 1 gm. kidney tissue per twenty-four hours, in the dog. Urea administered to rabbits in quantities of 0.1-0.2 per cent. of the total body weight evokes severe disturbance and death.

(4) *A Regulator of Osmotic Pressure.*—Urea is too soluble and diffusible to have an appreciable local effect on the osmotic pressure of the mammalian organism; and even when produced in quantity during protein metabolism, its removal by the kidney is so rapid that it can have little effect on the general osmotic pressure of the animal. The elasmobranchs, or cartilaginous fishes, such as the skate, dogfish and the shark, are unique in possessing in their blood, body fluids and tissues, concentrations of urea as high as 2-3 per cent. This physiological hyper-uræmia is due to the relative impermeability of the gills and the integument, and to the active conservation of urea by the elasmobranch kidney. As W. H. Smith has shown (1936), under these conditions, urea acts as a factor in the regulation of the osmotic pressure, and renders the elasmobranch osmotically superior to its environment, and able to absorb a sufficient quantity of water from its marine environment to provide for the secretion of urine. Marine teleosts, or bony fishes, do not conserve urea, and obtain their necessary water supply by drinking sea water and excreting through their gills a solution having a higher sodium chloride content than that of their environment.

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CHAPTER 23

EXCRETION

The higher organism returns matter to the environment through four channels: the lungs, the skin, the intestine and the kidneys. Gaseous waste-products, notably carbon dioxide and water vapour, escape by the lungs; water and about 1 per cent. of solutes are lost in the perspiration; insoluble salts, food residues, mucin, and lipides are excreted by the intestine; and soluble salts and organic end-products are excreted in the urine.

From the clinical aspect, urine is most significant, since (with the exception of some ammonia and hippuric acid) every constituent has been derived from the blood, and has at one time formed part of the internal environment of the organism.

Average Composition of Normal Human Urine Solute excreted in Twenty-four Hours

Total volume	1,250 ml.	pH 6.2.
Total solids	58 gm.	
Total nitrogen	17 gm.	Depends chiefly on protein intake.
Urea	28 gm.	
Creatinine	1.5 gm.	Independent of normal diet.
Uric acid	0.7 gm.	Partly independent of diet.
Methyl purines	0.1 gm.	Dependent on tea and coffee intake.
Hippuric acid	0.65 gm.	Increased by fruit diet.
Amino acids	+	Increased in wasting diseases.
Ammonia	0.7 gm.	Increased in acidoses.
Indoxyl sulphate	0.01 gm.	Depends on intestinal putrefaction.
Chloride, as Cl	7 gm.	Depends on salt intake.
Phosphate, as H_2PO_4 . .	2.6 gm.	Depends on phosphoproteins of diet.
Sulphate, as H_2SO_4 . . .	2.0 gm.	Depends on proteins of diet.
Sodium	5 gm.	
Potassium	1.6 gm.	Increased by vegetable diet.
Calcium	0.2 gm.	
Magnesium	0.2 gm.	Increased by vegetable diet.
Iron	1.5 mg.	
Sugar	0.7 gm.	Mostly pentose.
Oxalate	0.03 gm.	Depends on diet and gastric fermentation of sugar.
Citrate	0.5 gm.	
Lactate	+	Increased by muscular activity.
Thiol compounds	0.3 gm.	
Pigments	+	
Steroids	+	

The composition of the urine varies during the day, owing to alterations in metabolic activity, and for comparative purposes the

twenty-four-hour output is taken as being representative. The extent to which individual variation can occur is shown in the following table compiled from data by Powell White (1925), who has studied the correlations of the various solutes.

*Composition of Urine from 50 Normal Subjects
Expressed in mg. per 100 ml.*

Solute.	Average.	Maximum.	Minimum.
Urea N . . .	682	1,829	298
Urea . . .	1,459	3,914	638
Creatinine N . . .	36	90	17
Creatinine . . .	97.2	243	45.9
Uric acid N . . .	12.3	30.7	3.7
Uric acid . . .	36.9	92	11
Amino N . . .	9.7	42.5	0.6
Ammonia N . . .	57	189	13
Sodium . . .	212	608	46
Potassium . . .	137	245	56
Calcium . . .	19.5	72.5	0.6
Magnesium . . .	11.3	23.9	2.7
Chloride . . .	314	579	99
Total sulphate . . .	91	326	34
Inorganic sulphate . . .	83	304	32
Organic sulphate . . .	5.3	25.6	0.6
Inorganic phosphate . . .	179	426	77
pH . . .	6.4	8.2	5.8
Total acidity as ml. N/10 acid . . .	27.8	76.8	4.0

Apart from tests for pathological constituents, analysis of random specimens of urine is of very little value. Samples should be taken from the mixed total twenty-four-hour output of known volume, and results should be expressed in terms of (1) percentage, and (2) quantity excreted *per diem*.

HUMAN URINE

(1) **Total Volume in Twenty-four Hours.**—Adult, 950–1,500 ml. (33–60 oz.); child, 400–600 ml. Night urine is about one-quarter to one-half the volume of day urine.

The total volume varies greatly with diet and season of the year, the average secretion rate during the day being 1 ml. per minute.

In polyuria, such as diabetes insipidus due to lack of the anti-diuretin autacoid of the pituitary gland, the daily output of urine may be as great as 10 litres, and is balanced by a corresponding thirst. In oliguria, the output may be only a few ml., or nil in complete suppression (anuria).

(2) **Specific Gravity.**—1,015–1,020 at 15° C. (water = 1,000). This is a measure of the concentration of urinary solutes, and is inversely related to the total volume. After drinking an excess of water, the specific gravity may be as low as 1,002; whereas, after twelve hours' abstention from fluids, it may rise to 1,030–1,035.

In finding specific gravity, the urine should be at room temperature (15° C.), and the hydrometer floating freely in the liquid. Froth on the surface can be removed by a drop of alcohol.

(3) **Appearance.**—Fresh urine is usually transparent, but may be opaque after meals owing to the "alkaline tide" causing a precipitation of calcium and magnesium phosphate (p. 395). As urine cools, a cloudy suspension of mucoprotein from the urinary tract may appear in the body of the fluid.

(4) **Colour.**—The normal colour of urine is due chiefly to two pigments :—

(a) *Urochrome*, a stable yellow pigment invariably present in urine (p. 206). It has no characteristic spectrum, and its excretion has not been correlated with any particular metabolic condition. Urochrome can be obtained in a very impure form by extracting acidified urine with amyl or butyl alcohol.

(b) *Urobilin*, a brown pigment excreted as a colourless precursor, urobilinogen, which gradually oxidises on exposure to light and air. Urines rich in urobilinogen darken on standing owing to this spontaneous oxidation.

Urobilinogen is derived from bilirubin that has undergone reduction in the intestine, and its excretion is an index of the degree of intestinal stasis and putrefaction. It disappears from the urine in obstructive jaundice, being replaced by bilirubin excreted directly from the plasma, but it reappears in an increased amount during the recovery stage. Urines containing urobilinogen give a red colour on addition of excess of an acid solution of Ehrlich's aldehyde reagent (p. 166), without application of heat. Urobilin does not give this reaction, but may be detected by its characteristic spectrum (p. 206), and by the green fluorescence developed on addition of

excess of a 2 per cent. solution of zinc acetate in alcohol. Minor pigments present in urine are *uroerythrin*, which imparts a red colour to uric acid deposits, and *uroporphyrin*, a solute greatly increased in the congenital condition of porphyria.

(5) **H-ion Concentration.**—Urine is usually slightly acid in reaction, the average value being pH 6.0, but it may vary from pH 4.8–pH 7.5 during the twenty-four hours. After rising in the morning, and about half an hour after each meal, the reaction of urine tends to shift towards alkalinity, the so-called “alkaline tide.” This promotes the precipitation of calcium and magnesium phosphates.

The H-ion concentration of urine may be determined electrometrically or colorimetrically by means of selected indicators covering the range pH 4–pH 8.

(6) **Total or Titration Acidity.**—This is found by titrating 10 ml. of urine with N/10 NaOH, using 5 drops of 0.1 per cent. phenol-red or phenolphthalein as indicator. The result is expressed as the quantity of N/10 NaOH required to neutralise (1) 100 ml. urine, or (2) the total volume of urine excreted in twenty-four hours.

Titration is a measure of the available acidity of urine; this depends chiefly on the concentration of acid phosphate ions, and also on the uric and hippuric acid present. Usually 200–500 ml. N/10 NaOH are required to neutralise the acidity of an entire twenty-four hour sample, using phenol red (end-point, pH 7.7–7.5); and 300–800 ml. N/10 NaOH, using phenolphthalein (end-point, pH 8.5–9).

To determine the end-point, compare the urine being titrated with an untitrated specimen in a similar test tube. As soon as a colour difference can be detected the titration is complete. Shortly before the end-point is reached, a precipitate of calcium and magnesium phosphate may appear in the solution. This is due to the conversion of soluble acid phosphate into insoluble phosphate by action of the alkali. Folin advocates the precipitation of the calcium by the addition of neutral potassium oxalate before starting the titration.

INORGANIC SOLUTES

Ammonia.—The average percentage of ammonia is 0.05, depending on the N in the diet. It is present as NH_4^+ , unless the urine has undergone fermentation, when free ammonia may be detected. Urinary ammonia comes from glutamine deamination in the kidney tissue (Van Slyke *et al.*, 1943), in response to acidosis, being part of the neutrality-maintaining mechanism of the organism.

Tests for Ammonia.—(1) *Nessler's Reaction.*—To 5 ml. of water add a few drops of Nessler's reagent. There is no colour change if the water be free from ammonia. Now add a drop of urine. An intense yellow colour develops owing to the ammonia present in the urine. (2) Heat 5 ml. of urine with a little solid sodium carbonate. Ammonia is set free, and can be detected by the smell and by the bluing of red litmus paper held above the tube.

Estimation of Ammonia.—Neutralise 10 ml. of urine to phenolphthalein, using N/10 NaOH, as in the estimation of the total acidity of urine. Neutralise 2–3 ml. of commercial formalin solution (30–40 per cent. formaldehyde) in the same manner. Mix the two neutral solutions. The mixture becomes acid, and the pink colour of the indicator is discharged. This is because the H-ions previously combined with the ammonia have been liberated by the formaldehyde. Titrate this increase in acidity as before with N/10 NaOH. Each ml. of alkali required corresponds to 1.7 mg. of NH_3 in the original urine. The ammonia value obtained by the formaldehyde method is usually 10–25 per cent. too high owing to the fact that the $-\text{NH}_2$ groups of other urinary solutes also interact. The discrepancy is usually neglected in clinical estimations.

Urinary ammonia may be estimated accurately by (a) aspiration and (b) by colorimetry, using Nessler's reagent.

The average ammonia output in twenty-four hours is about 0.7 gm., corresponding to 400 ml. N/10 NaOH. It is increased in conditions of acidosis, and by dietaries rich in protein.

Chloride.—Next to urea, chloride is the chief solute of urine. Expressed as NaCl, the average daily excretion is 10–15 gm., and the urinary concentration is about 0.7–1.0 per cent.

Silver Test for Chloride.—Add 1 ml. of 1 per cent. AgNO_3 to 5 ml. of urine. A whitish precipitate forms, made up of silver chloride, carbonate, and phosphate. Acidify with about 10 drops of concentrated HNO_3 . Carbonate and phosphate dissolve, leaving a white residue of AgCl.

Estimation of Chloride by the Tartrazol Method.—Transfer by means of a pipette 10 ml. of N/10 AgNO_3 to a 100 ml. measuring cylinder. Acidify with 5 drops of concentrated HNO_3 . Add 2–4 drops of 0.5 per cent. tartrazine (tartrazol, or "tartar yellow"). Titrate with the urine, or other chloride solution, from a burette, by additions of 0.2 ml. at a time. Shake the yellow mixture vigorously after each addition of chloride. The first effect of the chloride is to form a white precipitate of silver chloride, this rapidly adsorbs the indicator, and settles down as a buff-coloured deposit. The surrounding liquid becomes colourless owing to removal of the tartrazol.

The end-point is reached when all the silver has been precipitated as chloride. At this stage, addition of a slight excess of chloride causes a sudden release of the adsorbed indicator. The colourless solution turns bright yellow, and the silver precipitate changes from buff to pure white. The estimation should be done in duplicate as the end-point is very sharp, and may be overshoot the first time.

Calculation.—Since 10 ml. N/10 AgNO_3 = 58 mg. NaCl, $x = \frac{5.8}{n}$,

where n = number of ml. of chloride solution added,

and x = percentage of chloride, expressed as gm. NaCl per 100 ml.

The usual value for n is 5–9, corresponding to an NaCl content of 1.16–0.63 per cent.

If the chloride content of the urine is less than 0.1 per cent., use 1 ml. of N/10 AgNO_3 in a test tube, and add 1 drop of indicator.

Proteins in pathological urine interfere with the end-point by inhibiting the aggregation of the AgCl , but do not affect the colour change.

Phosphate.—(a) *General Test.*—Acidify 5 ml. of urine with about 5 drops of concentrated nitric acid. Add 2 ml. of 2 per cent. ammonium molybdate ($(\text{NH}_4)_2\text{MoO}_4$), and boil gently. A bright yellow precipitate of phosphomolybdic acid denotes phosphate. Cool, and make just alkaline with NaOH. A deep blue colour forms, owing to reduction of the phosphomolybdate by the uric acid of the urine.

(b) *"Earthy Phosphates."*—Make 5 ml. of urine alkaline with about 10 drops of ammonium hydroxide. A cloudy precipitate of calcium and magnesium phosphates ("earthy phosphates") forms. Heat the mixture. The precipitate flocculates. Acidify with 20 per cent. acetic acid. The precipitate dissolves.

The precipitation of these phosphates when urine is boiled is a common fallacy in the heat-coagulation test for proteins in urine. They can be distinguished from proteins by their solubility in acids. Earthy phosphates tend to precipitate whenever the H-ion value of urine falls below pH 6, and thus they appear during the "alkaline tide" after meals.

Estimation of Phosphate.—Urinary phosphate may be rapidly and roughly estimated by titration with uranium acetate. Accurate colorimetric methods, suitable for blood filtrates as well as urine, depend on the formation of phosphomolybdate, and its subsequent reduction to "molybdenum blue" by hydroquinone or other appropriate reagent.

The daily phosphate output, expressed as the acid, H_3PO_4 , is

about 2.6 gm. It is largely derived from the phosphoproteins of the diet.

Sulphate.—(a) *Inorganic.*—Add a few drops of 2 per cent. BaCl_2 to 5 ml. of urine. A white precipitate of BaSO_4 forms. This does not dissolve on addition of strong nitric acid, thus being distinguished from BaCO_3 and $\text{Ba}_3(\text{PO}_4)_2$, which are also precipitated on addition of BaCl_2 .

(b) *Organic.*—Add an excess (2–3 ml.) of BaCl_2 to 5 ml. of urine. Filter off the precipitated sulphate, carbonate, and phosphate, repeating the filtration, if necessary, to obtain a clear filtrate. Acidify the filtrate with a few drops of nitric acid, and boil for a few minutes. Gradually a second precipitate of barium sulphate forms, owing to the hydrolysis of various organic sulphates in the urine. These are compounds of indoxyl and other phenols, and are sometimes termed “ethereal sulphates.”

The total daily sulphate output, expressed as H_2SO_4 , is about 2 gm., and is derived from the sulphur-containing amino acids, cystine and methionine, of the protein dietary. About 80 per cent. of total sulphate is SO_4^{--} , and 20 per cent. is organic, representing the detoxication of phenols produced in the digestive tract.

Urinary sulphur also appears in a third form, sometimes termed “neutral sulphur.” This includes thiol compounds, such as CH_3SH , and thiocyanate, which do not interact with the barium reagent until they are oxidised to sulphate.

Calcium.—Acidify 5 ml. of urine with a few drops of glacial acetic acid, and add about 1 ml. of 5 per cent. potassium oxalate. A white, micro-crystalline precipitate of calcium oxalate gradually appears. When it has subsided, examine the sediment microscopically for the characteristic envelope-shaped crystals of the salt.

ORGANIC SOLUTES

Urea, $\text{H}_2\text{N} \cdot \text{C}(\text{NH}) \cdot \text{OH}$, the chief nitrogenous constituent of mammalian urine, is derived from the protein of the diet in the proportions of about 30 gm. urea per 100 gm. of protein. The daily output is 25–32 gm., and the urinary concentration is usually in the region of 2 per cent.

TESTS: (1) *Zymolysis by Urease.*—To 5 ml. urine add a little urease preparation (soy bean powder or extract) and 5 drops of phenol red or “universal” indicator. If the mixture be not acid to the indicator (i.e., yellow to phenol red at the start), carefully acidify with a weak acid (0.2 per cent. acetic). Incubate at 40–50° C. or keep at room temperature. The presence of urea is

shown by the mixture gradually becoming alkaline owing to liberated NH_3 .

(2) *Decomposition by hypobromite*.—To 5 ml. urine add about 1 ml. fresh HBrO reagent. The urea is decomposed with liberation of free N_2 , and the mixture effervesces vigorously. Ammonium salts, amino acids, and other compounds containing the *amino group* $-\text{NH}_2$ are decomposed in the same way.

(3) *Diacetyl Test*.—Apply this test to 2–3 drops of urine mixed with 2–3 ml. concentrated HCl (p. 441).

Estimation of Urea.—(a) *Decomposition by Urease*.—This is employed in the standard methods for the accurate estimation of urea in blood, urine and other biological liquids. The urea is converted into ammonium carbonate, which is estimated directly by Nessler's reagent, or aspirated into standard acid (Marshall and Van Slyke, 1915), or allowed to diffuse into standard acid (Conway, 1933).

(b) *Decomposition by Hypobromite*.—By measuring the nitrogen evolved as gas when urea is decomposed by hypobromite in presence of alkali, which fixes the carbon dioxide, a value is obtained which is sufficiently accurate for clinical estimations. Many different types of apparatus are in use.

Hypobromite Reagent.—Add 3 ml. (\equiv 8 gm.) of bromine slowly to a cold solution of 8 gm. NaOH in 25 ml. H_2O . The mixture contains 98 per cent. of the theoretical NaBrO , and 3 ml. will decompose 60 mg. urea. The method is convenient but inexact, as the NH_4^+ and amino acids also liberate N_2 when treated with alkaline BrO^- . This is partly compensated by the failure of the reagent to liberate all the nitrogen from urea. In practice, it is assumed that 1 ml. of N_2 evolved from urine, at N.T.P., represents 2.8 mg. urea.

Uric Acid, $\text{C}_5\text{H}_4\text{N}_4\text{O}_3$, the least soluble organic constituent of urine. Average value, 0.04 per cent., or 0.6–0.9 gm. in twenty-four hours. It is an end-product of nucleoprotein metabolism.

Phosphotungstate Test.—Saturate 5 ml. urine with sodium carbonate, or add 10 drops of urine to 10 ml. saturated sodium carbonate solution. Then add about 1 ml. of Folin's uric acid reagent; an intense blue colour develops, approximately proportional to the amount of uric acid present.

In this form, the test is not selective, as other urinary solutes, such as thiols and vitamin C, also reduce the reagent.

Chloroimide Test.—Add about 10 drops of urine to 5 ml. of water so as to obtain a nearly colourless solution. Add 2 drops of the reagent (0.4 per cent. alcoholic 2:6-dichloro-quinone-chloroimide). Make the mixture slightly alkaline (pH 8–10) by

addition of about 0.5 gm. solid sodium acetate or 2-5 drops of N/10 NaOH. A bright yellow colour develops depending on the concentration of uric acid present.

The test will detect uric acid in calculi and urinary deposits, and in saliva, and can be adapted for colorimetric estimation. None of the other common purine derivatives react, though colours (red to blue) are given by free phenols, and some amino acids, but these do not occur in sufficient quantities in urine to interfere with the test.

Creatinine, $C_4H_7N_3O$, the anhydride of creatine. A constant and characteristic constituent of mammalian urine. The adult daily output is about 1.2 gm., and is independent of the diet.

TESTS: (1) *Picric Acid*.—Add 5 drops of saturated picric acid solution to 5 ml. urine. Make alkaline with a few drops of 20 per cent. NaOH. A deep orange colour develops proportional to the amount of creatinine present.

(2) *3,5-Dinitrobenzoic acid*, similarly applied, gives a purple colour with creatinine in alkaline solution. The reagent is more selective than picric acid.

(3) *Nitroprusside*.—Add 5 drops of fresh 5 per cent. sodium nitroprusside solution to 5 ml. urine. Make alkaline with a few drops of 20 per cent. NaOH. A deep ruby colour develops if creatinine be present. Acidify with 20 per cent. acetic acid; the colour is discharged.

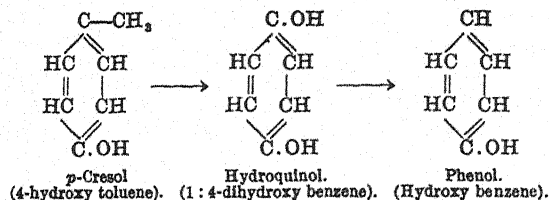
Note.—Acetoacetic acid and acetone, the pathological solutes found in ketosis, give a similar reaction with alkaline nitroprusside, but the colour is not discharged on acidification with acetic acid.

Hippuric acid, or benzoyl glycine, $C_6H_5.CO.NH.CH_2.COOH$, is synthesised in the kidney and in the liver from benzoic acid and glycine. The adult daily output is about 0.7 gm., being derived mostly from benzoic precursors in the vegetable diet.

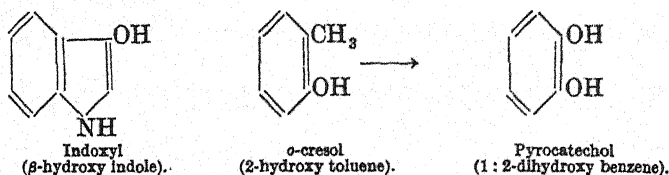
Urine is concentrated to one-quarter of its volume, filtered, acidified with H_2SO_4 and treated with $(NH_4)_2SO_4$ so as to make a 3 per cent. solution. On standing for twenty-four hours, hippuric acid crystallises out in four-sided prisms, which may be purified by recrystallisation from hot water. A good yield is obtained from the urine of herbivora, such as the horse or the cow.

Phenols.—Aromatic hydroxy compounds mostly derived from tyrosine and tryptophane putrefaction occur in the urine in normal health. The adult value on a mixed diet ranges from 20-60 mg. *per diem*. but is greatly increased in intestinal stasis and excessive protein dietaries.

(a) *Phenols derived from phenyl alanine and from tyrosine :—*

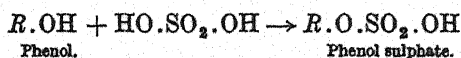


(b) *Phenols derived from tryptophane :—*



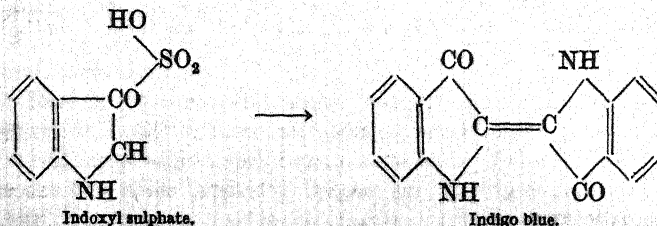
Various intermediate products derived from tryptophane have been reported as occasional urinary constituents. These include indole aldehyde, indole acetic acid, scatoxyl, and indole carboxylic acid. Indole and scatole, themselves, are improbable urinary constituents.

Urinary phenols are excreted as esters of sulphuric and glycuronic acids, in which form they are non-toxic.



(a) *General Test for Phenols.*—To 5 ml. of urine add Millon's tyrosine reagent drop by drop as long as a white precipitate forms. The precipitate settles out, and slowly turns red owing to the interaction between the adsorbed phenols and the reagent.

(b) *Indigo Test for Indoxyl.*—To 5 ml. of urine add an equal volume of concentrated HCl, and a drop of dilute (3 per cent.) hydrogen peroxide. Shake well. If the urine contains an excess of indoxyl it will be converted into indigo blue, and the urine will turn a dark bluish colour. Traces of blue may be detected by extracting the urine with chloroform, which dissolves the pigment.



Three stages are recognisable in this curious test : (i.) hydrolysis of indoxyl sulphate and liberation of free indoxyl by the action of the acid ; (ii.) union between two molecules of indoxyl to form the leuco-base, or precursor of indigo blue ; (iii.) oxidation of the leuco-base to form the pigment. If the oxidiser be in excess a fourth stage terminates the reaction, namely, (iv.) oxidation of the indigo blue to the almost colourless isatin.

The test is unreliable, and sufficiently insensitive to be a controversial method for detecting abnormal amounts of indoxyl in urine.

In the absence of an oxidising agent the blue colour will not develop ; in the presence of an excess of oxidiser the blue colour will be destroyed. Concentrated HCl has varying oxidising properties according to the amount of decomposition that has taken place owing to exposure to light. A yellowish specimen that contains oxides of chlorine is usually sufficiently active to give the indigo reaction without addition of H_2O_2 .

Many constituents in urine interfere with the test by combining with the liberated indoxyl.

(c) *α -Naphthol Test for Indoxyl.*—To 1 ml. of urine add 2–4 drops of 2 per cent. α -naphthol (or 2 per cent. resorcinol, or 2 per cent. thymol), and 10 ml. of concentrated hydrochloric acid. Shake well. If a colour does not appear in a minute, add a drop of 3 per cent. hydrogen peroxide. The liberated indoxyl combines with the α -naphthol to form a deep blue pigment (with resorcinol and with thymol, the colour of the pigment is carmine). This test requires 10–15 minutes to develop, and is so delicate that all specimens of normal urine respond more or less.

PATHOLOGICAL CONSTITUENTS OF URINE

While a discussion of the various pathological constituents of urine and their significance is outside the scope of this book, the occurrence and identification of several of them presents features of biochemical interest, and they will be described briefly.

Pathological constituents may be divided as follows :—

(a) Tissue components and metabolites that normally do not appear in the urine.

Examples are serum albumin and serum globulin, hæmoglobin, bile pigments, bile salts, blood sugar.

(b) Abnormal or exceptional metabolites that escape by the urine.

Examples are β -hydroxy-butyric acid, acetoacetic acid, acetone, homogentisic acid, Bence-Jones' protein, pentoses, lactose, methæmoglobin, uroporphyrin.

(1) *Proteinuria.*—Normal urine contains a trace of protein, probably derived from the urinary tract. Under abnormal renal conditions the serum proteins may appear, giving rise to **albuminuria**. Again, in disease of the kidney and other tissues, protein decomposi-

tion products may escape and cause *proteosuria*. While in multiple osteo-myelomata, skeletal material may be excreted as "Bence-Jones' protein."

Usually, however, the term *proteinuria* denotes *albuminuria*, the appearance of a heat-coagulable protein in the urine.

TESTS: (1) *Heat coagulation*.—Transfer about 5 ml. of urine to a test tube, and carefully boil the upper layer. If the urine contains albumin a white coagulum may appear in the heated part of the tube. This must not be confused with the white, cloudy precipitate of calcium and magnesium phosphates ("earthy phosphates") that forms when most specimens of normal urine are boiled. To distinguish, acidify the urine with a few drops of dilute acetic acid. The phosphate precipitate dissolves completely, the protein coagulum remains.

Urine that has become strongly alkaline owing to ammoniacal fermentation must be acidified slightly with acetic acid before the presence of protein can be detected by the heat-coagulation test.

Heat coagulation requires slight acidity and an electrolyte. Normal urine may provide the latter by its 1 per cent. content of NaCl, but urine from subjects of chloride retention may be so deficient in NaCl that any protein present will not coagulate on heating. To avoid this serious fallacy the modified form of the heat coagulation test has been devised, and should be used in all routine urine analysis.

(2) *Modified Test*. To 10 ml. urine add 1 ml. of the protein reagent (12 per cent. sodium acetate in 5 per cent. acetic acid), and boil for thirty seconds. If the urine remains clear, either it contains no coagulable protein or less than 5 mg. per 100 ml.

Bence-Jones' Protein occurs in the urine in conditions of multiple myeloma of bone marrow, in myeloid and lymphatic leucæmia, and, occasionally, after severe fractures. The protein has been obtained from bone marrow by Meyler (1936), who suggests that it is in some way associated with the growth and activities of the leucocytes.

This form of *proteinuria* was recognised by Bence-Jones by the fact that on heating the urine the protein began to flocculate at the relatively low temperature of 40° and re-dissolved on heating to 100°. On cooling, the precipitate reappears, and persists.

(3) *Nitric Acid Precipitation* (Heller's Test).—Place 2–3 ml. of concentrated nitric acid in a test tube and carefully add about 5 ml. of urine by means of a pipette so as to form a layer above the denser acid. An opaque white ring or cloud at the junction of the liquids denotes albumin.

(4) *Salicyl Sulphonic Acid Precipitation*.—To 3 ml. of urine add about 6 drops of 20 per cent. salicyl sulphonic acid. If the urine contains more than 0.02 per cent. of albumin a white cloud forms

almost at once and becomes more dense. Large quantities of protein give an opaque white precipitate.

The modified heat coagulation test and the salicyl sulphonic acid test are each about four times as sensitive as the nitric acid test.

Positive results with these tests do not differentiate between albumin and globulin in the urine. This can be done by fractional precipitation by neutral salts, but the distinction has not yet been shown to have clinical significance. Globulin sometimes accompanies albumin in albuminuria, though the ratio may vary.

Note.—All three protein tests should be applied in the routine examination of clinical specimens. To detect a faint precipitate in any one of the tests, hold the tube against a dark background, and compare the contents with some of the untreated specimens.

(2) *Hæmaturia.*—Blood may appear in the urine in the form of corpuscles, free hæmoglobin (hæmoglobinuria), and abnormal derivatives (methæmoglobin, and uroporphyrin). In hæmaturia, the urine often has a smoky, reddish colour that is very characteristic. Microscopic examination of the sediment may show the presence of blood corpuscles if they have escaped hæmolysis.

TESTS: (1) *Benzidine Test.*—Add about 10 drops of a fresh, strong solution of benzidine in glacial acetic acid to 1 ml. of 3 per cent. (10 vol. O₂ per 100 vol.) hydrogen peroxide. Add 1 ml. of urine, drop by drop. If blood be present the mixture turns blue.

Excess of urine must be avoided, as the sulphate present may precipitate the benzidine before the oxidation pigment has had time to form.

(2) *Guaiacum Test.*—Dissolve a small fragment of guaiacum resin in about 3 ml. of alcohol, with the aid of heat. Cool. Add 10 drops of 3 per cent. hydrogen peroxide and 1 ml. of urine. Mix well. If blood be present the mixture turns blue.

(3) *Pyramidon Test.*—Mix about 2 ml. of urine with an equal volume of 5 per cent. pyramidon (amidopyrine) in alcohol. Acidify with 2 drops of glacial acetic acid. Add 2–4 drops of 3 per cent. hydrogen peroxide. If blood be present, a lilac colour develops.

Notes.—These oxidation tests for blood depend on the presence of the heat-stable catalyst hæmoglobin, which they will reveal in dilutions as low as 1:10,000 to 1:50,000 in urine, and less than 1:200,000 in water.

A heat-labile catalyst, or enzyme, occurs in leucocytes, fresh milk and unboiled plant extracts, and will give a positive reaction with the reagents used in testing for blood. To distinguish, boil a sample of the urine, and repeat the test after cooling. If the response is no longer positive it was originally due to an enzyme.

The presence of leucocytes or pus in urine (pyuria) is most easily confirmed by microscopical examination of the sediment.

Urine after the administration of iodides usually gives a positive reaction with the blood reagents, and this may readily be mistaken for hæmoglobin as both reactants survive boiling previous to testing.

To distinguish, apply the spectroscope, and examine for absorption bands of blood pigment; and also centrifuge the specimen, and examine microscopically for un hæmolyzed red corpuscles.

(4) *Spectroscopic Test*.—This may require special treatment of the urine, as the concentration of pigment is often insufficient to show the characteristic absorption spectra (p 215).

The urine is acidified with acetic acid and extracted with an equal volume of ether. This dissolves out the hæmatin, which may be extracted with dilute ammonium hydroxide, and identified spectroscopically.

Hæmaturia is a serious condition, and the analysis for blood in the urine should be confirmed by more than one test.

(3) *Biliuria*.—Bile constituents may appear in the urine in obstructive jaundice (overflow biliuria), toxic jaundice, and various hæmolytic disorders leading to the decomposition of hæmoglobin.

In simple obstructive jaundice bilirubin appears, accompanied later by the bile salts. In the other forms of biliuria, pigments are present unaccompanied by bile salts.

Bile Pigments

Urine containing bile pigment (bilirubin) has a characteristic golden-brown colour and shows a transient yellow froth on being shaken.

(1) *Iodine Test*.—To 5 ml. of urine carefully add about 10 drops of 1 per cent. iodine in alcohol (tincture of iodine) so as to form a layer on the surface. A green ring gradually develops between the layers if the urine contains bilirubin. The test may also be carried out by putting a drop of iodine solution on a drop of urine spread on a filter paper.

(2) *Turpentine Test*.—Acidify 5 ml. of urine with about 10 drops of glacial acetic acid. Add 2 ml. of turpentine, shake so as to form an emulsion, and warm gently. Do not boil, or the mixture may spurt.

If bile pigment be present, the white turpentine emulsion gradually turns green. Eventually a layer of turpentine separates out on top; this is colourless in simple obstructive jaundice, but is greenish in the toxic and hæmolytic forms of biliuria.

The reason appears to be that in obstructive jaundice the overflow pigment appears in the urine in an esterified form, insoluble in warm turpentine. In toxic jaundice this esterification has not taken place, and the pigment is turpentine-soluble.

Bile Salts

(3) *Sulphur Sedimentation* (Hay's Test).—Sprinkle the surface of 10 ml. of urine with some finely powdered sulphur. In normal urine the particles remain on the surface supported by the tension at the urine-air interface. If, however, the urine contain bile salts, the interfacial tension is reduced, and the sulphur particles gradually sink through the liquid. A control test should be done at the same time, using 10 ml. of water.

(4) *Peptone Precipitation* (Oliver's Test).—Filter the urine, if necessary. Acidify 5 ml. with a few drops of glacial acetic acid, and add about 2 ml. of a clear 1 per cent. solution of peptone previously acidified with acetic acid. If bile salts be present a white precipitate forms.

These two tests illustrate two important properties of bile salts; reduction of surface tension, and precipitation of peptone. They are not as useful clinically as the tests for bile pigment.

(4) *Glycosuria*.—Normal urine contains small quantities of reducing sugars, the concentration being between 0.01 and 0.1 per cent., which is insufficient to affect the ordinary Benedict and Fehling tests. These sugars consist largely of pentoses and disaccharides, with a trace of glucose.

(1) *Benedict's Qualitative Test*.—Add 0.5 ml. of urine (8 drops, not more) to 5 ml. of Benedict's qualitative reagent. Mix, and heat over a small flame for one to two minutes, or, much better, immerse the tube in boiling water for two to three minutes (this avoids spurting of the mixture). Allow to cool for a few minutes, and observe the appearance.

A *positive result* is shown by a greenish turbidity with a yellow or red sediment. A slight yellow precipitate indicates 0.1–0.25 per cent. of sugar; a dense orange-red precipitate and a clear supernatant liquid indicates over 1.5 per cent. of sugar.

A *negative result* is shown by the solution remaining a clear blue, with possibly a grey precipitate due to excess of urates.

If excess of urine be added or if boiling be prolonged a positive result may be obtained with many specimens of normal urine owing to the traces of sugars they contain. For this reason, the technique of the test must be followed carefully.

A positive result with Benedict's reagent indicates the presence of one or more of the following pathological urinary constituents: glucose, lactose, pentose, fructose, glycuronic acid.

Glucose is by far the commonest reducing sugar found in urine; it may be identified by the osazone test, and by yeast fermentation, and verified by the observation of an accompanying hyperglycæmia.

Lactose is a normal constituent of most urines during the lactation period, and has no pathological significance. It can be distinguished from glucose by its non-fermentability by yeast, and by the methylamine test (p. 123).

Lactosazone in crystalline form can only be obtained with difficulty from urine containing lactose.

L-Xyloketose, the commonest urinary pentose, may be identified by the fact that it can reduce Benedict's reagent at temperatures much below boiling.

Reduction test for pentose and fructose (Lasker and Enklewitz, 1933): Mix 1 ml. of urine and 5 ml. of Benedict's qualitative reagent, and incubate at 55° C., for ten minutes. The appearance of a yellow precipitate indicates that the urine contains L-xyloketose or fructose. Fructosuria, which is very rare, may simulate glycosuria in that the urine gives a positive fermentation test and yields glucosazone.

Pentose in urine can also be detected by the aniline test (p. 126).

(2) *Fehling's Test*.—Boil equal volumes (3–5 ml.) of Fehling's mixed reagent and urine in separate tubes. If spontaneous reduction occur in the reagent, it must be discarded. When boiling, add the reagent in small quantities to the urine, and look for the colour change to orange that denotes reduction. The commoner fallacies of the test are described on p. 125.

(3) *Yeast Fermentation Test*.—The method is described on p. 127. Lactose, pentoses and glycuronic acid are the only non-fermentable copper-reducing substances likely to occur in urine, and the fermentation test should be used to check the tests of Benedict and Fehling.

(4) *Osazone Test*.—The phenylhydrazine reagent (p. 128) will readily detect glucose in urine down to concentrations of about 0.5 per cent., but is not so satisfactory in the detection of lactose, owing to the interfering effect of other urinary solutes.

Glycuronic acid, $\text{HOOC} \cdot (\text{CHOH})_4 \cdot \text{CHO}$, occurs in urine as a detoxication compound formed after the administration of chloral, camphor, naphthol, menthol, phenol, morphine, turpentine, anti-pyrin, aspirin, and other drugs, with which it is esterified.

The formation of glycuronates is regarded as a test of hepatic efficiency, and may be invoked by administration of 5 grains of aspirin by the mouth. Glycuronic acid reduces Benedict's and Fehling's reagents and is not fermentable by yeast.

Naphthoresorcinol Test (Tollens).—Add 5 ml. concentrated HCl and 10 drops of 1 per cent. alcoholic naphthoresorcinol to 5 ml. of urine. Mix, and heat to boiling. Boil for one minute, shaking at intervals. A red colour develops. Let the tube stand for five minutes, cool under the tap, and extract with 5–10 ml. of ether. If

glycuronic acid be present the pigment dissolves in the ether to form a purple solution showing two absorption bands, one on the D-line, and one to the right of it.

Many saccharides give red-violet pigments with the naphthoresorcinol reagent, but these are insoluble in ether (p. 126).

Steroids.—Normal urine contains varying amounts of the steroid hormones, usually in the form of soluble glycuronic acid derivatives. The output is greatly increased during pregnancy.

The 17-ketosteroid dehydro-*iso*-androsterone, and related androgens, occur in the urine in conditions of adrenal cortex disease, and are of value in diagnosis (Beaumont and Dodds, 1943). They give a purple colour with *m*-dinitrobenzene in strongly alkaline solution (Zimmermann's Test, 1935).

ABNORMAL METABOLITES IN URINE

(5) **Ketonuria.**—This term denotes the presence of one or all of the compounds, β -hydroxy-butyric acid, acetoacetic acid, and acetone. All are derived ultimately from fat metabolism, and appear in the urine when the carbohydrate available is insufficient to restrict the degradation of the fatty acids (p. 328).

There is no simple direct test for β -hydroxy-butyric acid, $\text{CH}_3\text{CH}(\text{OH})\text{CH}_2\text{COOH}$.

(1) **Iron Test for Acetoacetic Acid.**—Add dilute (1 per cent.) ferric chloride drop by drop to 5 ml. of urine until the buff-coloured precipitate of ferric phosphate, which is given by all specimens of urine, ceases to form. Further addition of the ferric chloride now produces a brown-purple colour if the urine contains more than 0.07 per cent. acetoacetic acid. Urines after administration of salicylates, aspirin, and related drugs, give a violet colour on addition of ferric chloride, which may be mistaken for an acetoacetic acid reaction.

To distinguish, boil a sample for five minutes, and repeat the test. Acetoacetic acid is converted into acetone by boiling and no longer gives a colour with ferric chloride; salicylates are unaffected, and still react.

(2) **Sodium Nitroprusside Test for Acetoacetic Acid and Acetone (Rothera).**—Fill up about 1 in. of a test tube with solid ammonium sulphate. Add 5 ml. of urine, and shake so as to saturate the mixture. Add 2–4 drops of fresh 5 per cent. sodium nitroprusside, and make alkaline with about 10 drops of strong ammonium hydroxide. A deep violet colour develops in a few seconds if the urine contains more than 0.2 per cent. acetoacetic acid, while 0.005 per cent. will give a pink colour in about ten minutes.

A similar colour is given by acetone, but the reaction is not so delicate, although it will reveal 0.01 per cent. in urine.

In dilute urines the colour appears first as a ring at the junction of the liquid and the crystals. The reaction should not be regarded as absolutely negative until the mixture has remained colourless for thirty minutes.

Normal urines rich in organic sulphides (thiols) give an immediate but transient red colour with the reagent. This must not be mistaken for a positive ketone reaction, which is stable for several hours, and violet in tint.

In the earlier form of the test, the ammonium sulphate was not used, but its presence greatly increases the sensitivity of the reaction.

Nitroprusside Reactions in Urine

Reagent.	Creatinine.	Acetone.	Acetoacetic Acid.
Na nitroprusside + NH_4OH Na nitroprusside + NaOH .	no change red (soon fades) ↓	purple red (stable) ↓	purple red (stable) ↓
Acidified with acetic acid .	decolourised	purple	purple
Na nitroprusside + NH_4OH + solid $(\text{NH}_4)_2\text{SO}_4$.	no change	violet	violet

Pyruvic acid, which may appear in urines when there is vitamin B_1 deficiency, develops a sapphire blue with Rothera's test.

(3) *Diffusion Test for Acetone*.—Place about 1 ml. of Nessler's reagent in a Conway unit or in a small watch-glass resting in a petri dish. Acidify about 2 ml. of urine with a drop of glacial acetic acid or hydrochloric acid. Carefully pour the urine into the dish around the watch-glass, taking care not to mix the liquids. Replace the cover on the petri dish. The presence of acetone in the urine is shown by the rapid appearance of a cream-coloured precipitate in the reagent in the watch-glass owing to the diffusion and fixation of the volatile acetone. This reaction is specific for acetone in urine.

(4) *Iodoform Test*.—Addition of 1–5 ml. of 1 per cent. aqueous iodine and 1–2 ml. 20 per cent. NaOH to 10 ml. of a urine containing acetone produces iodoform, CHI_3 , which is recognisable by its smell, and its separation as a pale yellow precipitate with a characteristic microscopic appearance (hexagonal plates and stars). Pyruvic and lactic acid, which are minor constituents of normal urine, give a

similar reaction, as also does alcohol, so the test is not reliable for detecting traces of acetone.

Ethyl Alcohol.—The detection of ethyl alcohol in urine may be important in the diagnosis of intoxications. Distil carefully 50–100 ml. of the urine, and apply the iodoform test and the nitro chromic test (p. 122) to 5 ml. samples of the distillate. Alcohol gives a positive reaction with both tests. Acetone, if present, will also come over in the distillate, and give a positive iodoform reaction, but does not give a blue colour with the nitro chromic reagent.

According to Newman (1942), only about 10 per cent. of ingested alcohol is excreted by the lungs and the kidneys; the rest is oxidised in the liver (p. 384). The alcohol level in the cerebro-spinal fluid and blood plasma is of chief diagnostic significance, though the resulting degree of intoxication depends on individual tolerance. An alcohol content of 225–275 mg. per 100 ml. plasma is toxic for nine-tenths of the community, while over 400 mg. is toxic for all.

ABNORMAL METABOLITES DUE TO INBORN DEFECTS

Alcaptonuria, characterised by the excretion of homogentisic acid. The urine darkens, owing to oxidative changes, after addition of alkalis or on undergoing spontaneous ammoniacal fermentation. It reduces alkaline copper reagents in an atypical way, and also gives a transient blue colour on addition of ferric chloride. The homogentisic acid arises from the phenylalanine and tyrosine of the diet. Related conditions are **Tyrosinosis** (p. 349) and **Phenylketonuria** (p. 351).

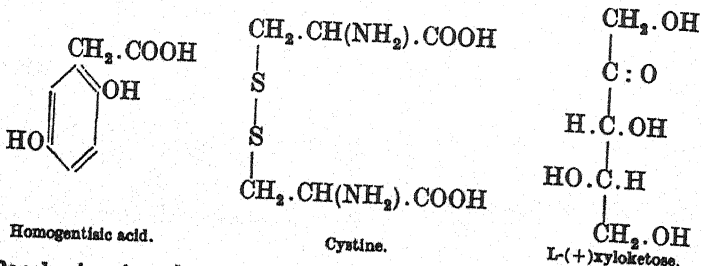
Phenylketonuria.—An example of a metabolic disorder associated with mental defect is found in the inherited condition of *imbecillitas phenylpyruvica*, in which phenylpyruvic acid is excreted in the urine. The output is increased by administration of phenylalanine but not by tyrosine.

Tyrosinosis.—An inability to metabolise all the phenylalanine and tyrosine of the diet, the surplus being excreted as tyrosine or its deamination product, hydroxyphenyl pyruvic acid.

Cystinuria, characterised by the excretion of cystine, which forms a crystalline deposit of hexagonal plates. The fresh urine has an aromatic smell resembling sweetbrier, and gives a black precipitate of lead sulphide on boiling with lead acetate and sodium hydroxide. The daily output is 1–2 gm., being derived from the cysteine and methionine of the dietary protein. Free cystine is not excreted as such by cystinurics, showing the metabolic error is in the breakdown of cystine compounds, which presumably appear as cystine

precursors in the urine. The diamines, cadaverine and putrescine often accompany cystine in cystinuria.

Pentosuria, characterised by the excretion of L-xyloketose, or other 5-carbon sugars, which on account of their reducing properties may be mistaken for glucose.



Porphyria, characterised by the increased production and excretion of uroporphyrins, which impart a red or brown colour to the urine. It may be evoked by lead, sulphonamides, and other agents capable of causing liver injury, and is distinct from the pathological **Porphyrias** (*Congenita* and *Acuta*), which are due to inborn defects. The accumulation of porphyrins in the blood may render the subject photo-sensitive to strong light.

Albinism, due to lack of tyrosinase and "dopa" oxidase, which form from tyrosine the natural melanin pigments of skin, hair and retina. The condition is unassociated with any abnormal urinary metabolite. Of these inborn errors, albinism is the most common, cystinuria is moderately rare, and the others are very uncommon. The most dangerous are porphyria, with its hypersensitisation to light, and cystinuria, which tends to the formation of renal calculi.

URINARY SEDIMENTS

According to the modern theory of solutions, dissolved salts are ionised, and consequently the electrolytes in urine are present as :

- I. *Cations* : Na^+ , K^+ , Ca^{++} , Mg^{++} , NH_4^+ .
- II. *Anions* : Cl^- , HPO_4^- , CO_3^- , SO_4^- , $\text{C}_5\text{H}_3\text{N}_4\text{O}_3^-$ (urate), $(\text{COO})_2^-$ (oxalate).

The appearance of a urinary sediment depends on (i.) temperature, (ii.) reaction, (iii.) relative concentration, and (iv.) absence of colloidal anti-precipitants. As secreted, all the urinary constituents are in solution at the temperature of the body, but when urine cools the urates and phosphates tend to separate out if the reaction be favourable. Urine is a better solvent for uric acid than water at the

same temperature and pH, a property that appears to be associated with the colloids present in small quantities.

(1) **Carbonates and Phosphates.**—Those of Na, K and NH_4 are freely soluble, and never form urinary precipitates. Those of Ca and Mg are soluble in acid urine ($\text{pH} < 6.5$) but insoluble in neutral or alkaline urine ($\text{pH} > 7.0$), and tend to precipitate when urine cools or becomes alkaline. Such precipitates are easily dissolved by acetic and other weak acids. Phosphatic calculi are among the commonest of the renal and bladder concretions.

(2) **Urates.**—Dibasic alkaline urates, such as $\text{Na}_2\text{C}_5\text{H}_2\text{N}_4\text{O}_3$, are soluble, and never occur as sediments, but the conditions for their existence demand a higher degree of alkali than that present in urine. Monobasic urates, especially ammonium dihydrogen urate, $\text{NH}_4\cdot\text{C}_5\text{H}_2\text{N}_4\text{O}_3$, are sparingly soluble in cold urine, but dissolve on heating or after addition of alkalis. They often appear, along with free uric acid, as an amorphous reddish sediment when urine cools, and are the most common and least important deposit.

(3) **Uric Acid** may accompany urates in very acid urine. Acidify 10 ml. urine with a few drops of strong HCl, and set aside for about twenty-four hours. The uric acid is precipitated as a few dark crystals, heavily pigmented. Remove by pipette and identify microscopically.

(4) **Oxalates** are soluble, with the important exception of calcium oxalate, which separates from acid or alkaline urine in octahedral crystals, and is a common source of calculi.

(5) **Chlorides and sulphates** are sufficiently soluble never to form urinary deposits or concretions

Urinary Sediments

Acid Urine.	Neutral or Alkaline Urine.
Free uric acid ("cayenne pepper" deposit).	Phosphates and carbonates of Ca and Mg (white, amorphous deposit).
Acid urates of NH_4 , Na or K ("brick dust" deposit).	Ammonium magnesium phosphate (triple phosphate) in ammoniacal urine. (Large, prismatic crystals.) Colourless.
Calcium oxalate, usually in very small amounts.	Ammonium urate.
("Envelope" crystals.)	Calcium oxalate.

DETOXICATION

Detoxication is a protective metabolic process whereby the animal modifies dangerous reactants, as when the toxic base, NH_3 ,

a latent end-product in amino acid degradation, is converted into a harmless, neutral solute, *urea*. The term *detoxication*, however, is generally restricted to the transformation of compounds foreign to the working-plan of the organism, such as phenols and cyclic bases, indole and scatole, liberated in the intestine by bacterial attack on amino acids. Other compounds excreted in detoxified form are drugs containing cyclic residues, such as the sulphonamides, and reagents, such as benzoic acid, given experimentally to reveal or test detoxication mechanisms.

Detoxication takes place chiefly in the liver. The process usually involves the masking of physiologically active polar groups, such as $-\text{OH}$, $-\text{CHO}$, $-\text{COOH}$ and $-\text{NH}_2$, attached to benzenoid and other cyclic nuclei resistant to tissue oxidation. The operation is effected by conjugation with glycuronic acid, glycine, glutamine, ornithine, acetic or sulphuric acid, or by methylation; according to the type of substrate and the species of animal concerned.

Glycuronic Acid.—D-glycuronic acid, $\text{HOOC}(\text{CH}(\text{OH}))_4\text{CHO}$, the commonest of the glycuronic acids, appears in urine in conjugated form after ingestion of benzoic acid, camphor, and many other drugs. On alkaline hydrolysis, the glycuronate is released and, being a reducing agent, gives all the ordinary copper-reducing reactions, and may be mistaken for sugar in the urine. Unlike the D-series hexoses, however, it is not fermented by yeast, and thus can be recognised. As Quick has shown (1937), two types of glycuronic derivatives can arise in detoxications: (1) *glycosides*, or *ethers*, formed by condensation of a phenolic $-\text{OH}$ with the C1 hydroxyl of glucopyranose, and (2) *esters*, formed by condensation of $-\text{COOH}$ with the C2 hydroxyl of the sugar.

Subsequent oxidation of the terminal $-\text{CH}_2\text{OH}$ converts the sugar derivative into a derivative of a glycuronic acid, in which form it is excreted in the urine. Glycuronic acids are relatively stable, and when administered to an animal are not conjugated with phenols, showing that the detoxication process employs glucose as the primary reactant. The extent to which glycuronic conjugation occurs in detoxication is determined by the supply of glucose available.

Quick has shown that a dog on an exclusive carbohydrate diet can produce 5 gm. of glucuronate in 24 hours, without an increase in nitrogen output. Insulin injection increases the capacity for producing glycuronate; if the hormone is absent, as in diabetes, or if the dietary carbohydrates are insufficient, the organism can utilise glucose collected from protein and other sources.

Glycine.—Aminoacetic acid is used freely for the detoxication of cyclic carboxyl groups, with which it combines by means of its

—NH₂ group, forming a peptide-type of linkage. *Hippuric acid*, or benzoyl glycine, derived from benzoic acid, is the best known of these products, being a normal solute in the urine of man and other higher animals. Conjugation is effected by the reversible action of the enzyme, *hippuricase*, which is found in liver and kidney of most animals, except the dog, in which it is restricted to the kidney. Quick (1938) has used the reaction as a test for hepatic function.

When 1.77 gm. of sodium benzoate in 20 ml. of water are slowly injected intravenously, the urine excreted during the following hour contains 0.7–0.55 gm. of hippuric acid, if the liver be working efficiently. Low values are found in hepatic cirrhosis, carcinoma and cholelithiasis.

The presence of a substituent group in the *ortho* position, adjacent to the carboxyl in benzoic acid, inhibits conjugation with glycine, a fact that may account for the therapeutic potency of ortho-substitution drugs, such as salicylic acid (2-hydroxybenzoic acid).

Glutamine, reacting through its α -amino group, is used similarly by man and apes, in the detoxication of phenylacetic acid, C₆H₅.CH₂.COOH, to *phenylacetyl glutamine*. Dogs and other mammals use glycine, forming thereby *phenylaceturic acid* (phenyl-acetyl glycine). Birds are exceptional among all the vertebrates examined, in that they employ ornithine instead of glycine for the detoxication of benzoic acid.

Acetic Acid.—Acetylation is not of value in detoxication of —OH groups, since acetylated hydroxy compounds, including drugs such as aspirin (acetylsalicylic acid), are readily hydrolysed in the animal. It is, however, the chief way in which cyclic amines are modified, although, as with the sulphonamides, the resulting acetyl-amine may be more toxic than the parent compound, and the process cannot be described accurately as a detoxication. Like glucuronic acid, acetic acid has no detoxicant value when administered, and must arise locally during a coupled metabolism involving sugar or fatty acid.

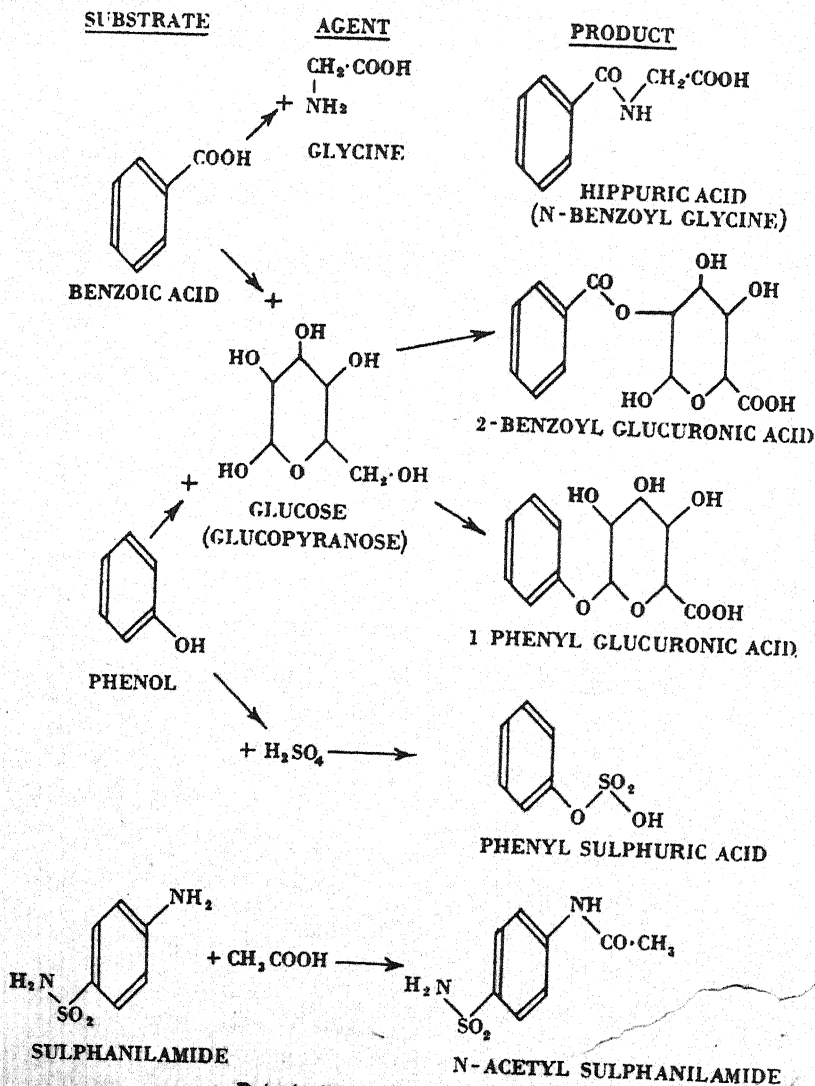
The urinary excretion of these acetylation products is of interest as providing the first direct proof that acetic acid can be formed in the course of animal metabolism.

Sulphuric Acid.—Many phenols, notably indoxyl, are excreted as mono-esters of sulphuric acid, forming the so-called organic sulphates of urine. Inorganic sulphate included in the diet can to some extent provide the acid radicle for the esterification (Hele, 1924), but the usual sources are the sulphur-containing amino acids.

Methylation.—By transfer of CH₃ from methionine and, possibly, choline, both pyridine and its important derived vitamin, nicotinic acid, are excreted as the tetravalent N compounds, methylpyridine

hydroxide, and trigonellin, respectively. Since nicotinic acid is necessary for life, its elimination in this inactive form is not, strictly, an example of a protective metabolic operation.

Methylation is a common event in plant metabolism.



Detoxication in the Human Body.

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CHAPTER 24

HORMONES

"Except during the nine months before he draws his first breath, no man manages his affairs as well as a tree does."

G. B. SHAW.

Hormones, or autacoids, are autogenous compounds manufactured and employed for the chemical co-ordination of the organism. They are specific chemical reactants produced by a tissue, and have the characteristic of exerting an effect of functional value on tissues or organs.

Classification.—The following classification is that proposed by Huxley (1935), with the exception that his use of the term *activator* to include all these chemical regulators has been changed in favour of the term *autacoid* (αὐτός, self; ἀκος, remedy), originally suggested by Schafer.

They are termed, alternatively, (i.) *internal secretions*, or endocrines, because many are sent directly into the blood stream from the tissue that produces them; and (ii.) *hormones*, from the fact that many but not all are physiological stimulants (ἐμπύω, I excite).

(1) **Local autacoids**, affecting the same cell or tissue in which they are produced.

(i.) *Intracellular hormones*.

(ii.) *Regional hormones*, which are responsible for the pre-determination of specific regions in the embryo, such as the limb-disc, the eye-rudiment and the chick pronephros.

(2) **Diffusion autacoids**, distributed by diffusion through the tissues.

(i.) *Growth hormones of plants*, such as the auxins.

(ii.) *Evocators and organisers* of the vertebrate embryo.

(iii.) *Neurocrines*, or chemical transmitters of the nerve impulse. Cholinergic and adrenergic factors.

(3) **Circulating hormones**, which are transported by the blood stream or lymph to all parts of the organism. These are represented by the endocrine secretions of the ductless glands.

(4) **Para-hormones**.—By-products of normal and pathological metabolism. Carbon dioxide, the regulator of the respiratory centre. Histamine, the regulator of capillary blood volume.

General Characters of Hormones.—(1) *High Potency*.—Autacoids

resemble many of the alkaloids and the vitamins in being effective in very small dosages.

(2) *Toleration*.—When autogenous, the autacoids do not evoke anaphylactic responses or immunity in the organism.

(3) *Relatively Low Molecular Weight and Simple Structure*.—This is true of adrenaline and histamine, but does not apply to the hormoproteins.

(4) *Thermal Stability*.—Unlike enzymes, most of the autacoids are not destroyed by boiling in neutral solutions. Decomposition may occur in presence of strong alkalies, acids, or oxidisers.

(5) *Biological Lability*.—Many autacoids are readily destroyed after they have entered the circulation, and, in consequence, have a temporary and local effect. Others, such as oestradiol, are excreted in the urine after slight modification.

(6) With few exceptions, autacoids are destroyed in the alimentary tract, and are ineffective when administered orally.

The existence of an autacoid is established in six different ways :—

(i.) Histological recognition of endocrine tissue. This led Schafer to conclude that an internal secretion was located in the islet tissue of the pancreas, and he suggested the name *insuline* many years before the actual autacoid was discovered.

(ii.) Pathological conditions associated with changes in endocrine tissue. Examples are Addison's disease (suprarenals), Graves's disease (thyroid), Fröhlich's syndrome (pituitary).

(iii.) Physiological response to administration of endocrine extracts.

(iv.) Isolation and identification of the active principle.

(v.) Production of characteristic pathological conditions by removal of endocrine tissue.

(vi.) Adequate compensation for endocrine deficiency by administration of autacoid, or gland extract, or by gland implantation.

Significance and Mode of Action.—Langdon Brown has suggested that the animal autacoids are the survival of the primitive chemical apparatus which regulated the organism before the evolution of a central nervous system, which, when it arose, became allied to the pre-existing endocrine system in two ways. The sympathetic mechanism became associated with the adrenal, thyroid and pituitary glands; the parasympathetic mechanism became associated with the insular tissue of the pancreas and with the choline bases.

The circulating hormones have been evolved in association with highly specialised tissues, which are represented in all vertebrates by the adrenal, thyroid, pituitary and sex glands.

Animal Hormones

Source.	Hormone.	Effect.
Adrenal cortex .	Desoxycorticosterone. Corticosterone. Dehydrocorticosterone. Hydroxycorticosterone. Hydroxydehydro- corticosterone. Adrenosterone. Progesterone. Adrenaline.	Promotes reabsorption of Na^+ and Cl^- by renal tubule. Glucotropic : promotes hepatic gluconeogenesis and glycogenesis. Androgenic. Progestational control. Sympathomimetic. Glucotropic. Promotes tissue oxidation. Promotes renal excretion of PO_4''' , bone decalcification, and hypercalcaemia. Promotes glucose utilisation. Promotes transport of fat from liver.
Adrenal medulla .		
Thyroid .	Thyroglobulin.	
Parathyroid .	Parathormone (Parathyrin).	
Pancreas .	Insulin. Lipocaeic.	
Ovarian follicle .	Œstradiol. Œstrone. Œstriol. Equilin. Equilenin.	Œstrogenic : maintains sex characteristics and induces oestrus cycle.
Corpus luteum .	Progesterone.	
Placenta .	Chorionic gonadotropin (APL principle) Œstriol. Œstriol glycuronide (Emmenin).	Progestational control of uterus ; placental development. Œstrogenic.
Testicle .	Testosterone. Androsterone. Dehydroandrosterone.	Androgenic : maintains male sex characteristics.
Kidney .	Renin.	Indirect vaso-pressor : liberates hypertensin in blood.

A. Adrenal Cortex

The adrenal gland is composed of two structures, independent in history and physiological function. The cortex is of mesoblastic origin, and arises from the coelomic epithelium on either side of the root of the mesentery ; the medulla arises from the primitive nerve tissue beside the posterior root ganglia, which subsequently differentiates into the sympathetic nervous system. Unlike the medulla, the adrenal cortex is necessary for life, and for this reason removal of the entire gland is fatal in animals not adequately equipped with accessory adrenal tissue.

Corticosterones.—By fractional extraction of adrenal cortex

Animal Hormones

Source.	Hormone.	Action.
Anterior pituitary.	Growth factor.	Promotes general growth; may stimulate protein metabolism.
	Thyrotropin.	Promotes thyroid activity.
	Corticotropin (Adrenotropin).	Promotes adrenal cortex activity.
	Ketogenic factor.	Promotes transport of fat to liver, and fat metabolism.
	Glycotropic factor (Pancreotropin) (diabetogenic principle).	Promotes sugar mobilisation and hyperglycæmia.
<i>Pars intermedia</i>	Follicle-stimulating factor, FSH.	Gonadotropic: promotes growth of ovarian follicle and seminiferous tubule.
	Luteinising factor, LH (Interstitial-cell-stimulating hormone, ICSH).	Promotes growth of corpus luteum, and secretion of sex hormones in female and male.
	Prolactin.	Lactogenic.
	Intermedin.	Promotes pigment-dispersion in melanophores.
Posterior pituitary.	Metabolic principle.	Promotes fat metabolism and retards sugar breakdown.
	Vasopressin (pitressin).	Vaso-constrictor, Anti-diuretic.
	Oxytocin (pitocin).	Promotes uterine contraction.
Gastric mucosa	Gastrin.	Promotes secretion of gastric juice.
Intestinal mucosa.	Secretin.	Promotes secretion of pancreatic juice.
	Enterogastrone.	Inhibits gastric motility and secretion.
	Cholecystokinin.	Promotes contraction of gall bladder.
	Villikinin.	Increases motility of villi.

with lipide solvents, Hartmann (1928), Swingle, and other workers, obtained an active preparation, *cortin*, later shown to be a mixture of steroids. All are derived from the androstane nucleus, and all carry $-\text{CO}-\text{CH}_2.\text{OH}$ as a side-chain at C17. Corticosterone and desoxycorticosterone are the most active of some thirty steroids separated from the cortex.

Effects of Cortical Deficiency.—(1) *Metabolic*.—Complete removal of the cortical tissue from an animal results in death within a week. There is rapid loss in weight, general muscular weakness, fall in temperature and reduction in basal metabolism by about 25 per cent. The syndrome is seen in a less acute form in partial removal

of the cortex, and in pathological dysfunction, as in Addison's disease and in vitamin B₂ deficiency.

By continued injection of cortin extracts, cats have been kept alive for a year after all cortical tissue had been removed.

Cortical deficiency, either due to Addison's disease or to experimental adrenalectomy, is shown by disturbance of three aspects of metabolism: (a) *carbohydrate utilisation*.—Sugar absorption from the intestine is retarded, the glycogen content of liver and muscle decreases, blood lactate increases owing to the inability of the liver to convert it into glycogen. Death is preceded by marked hypoglycaemia.

(b) *ionic balance*.—The output of Na⁺ and Cl⁻ in the urine is increased and the plasma Na⁺ level falls. This is partly compensated by migration of K⁺ from tissues to plasma.

(c) *dehydration*.—Water output is increased along with the increased loss of Na⁺ and Cl⁻. As a result, plasma volume decreases, and the blood concentration of haemoglobin and plasma proteins rises. These ionic disturbances can be rectified by administration of NaCl, without much improvement in the disturbance of carbohydrate metabolism. The problem is confused by the fact that some of the cortical steroids only affect carbohydrate metabolism, while others have mutually antagonistic effects on ionic balance.

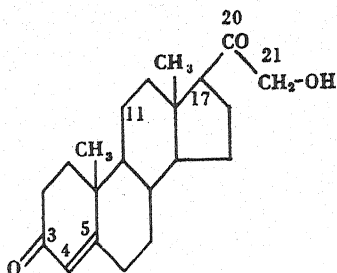
(2) *Androgenic*.—Dysfunction of the cortex owing to tumour formation in early childhood is associated with precocious sexual development in girls and boys. Dysfunction in adult females leads to the appearance of the secondary sexual characters of the male. From this it is inferred that an androgenic or masculine autacoid is being elaborated in the gland, and one such factor, *adrenosterone*, C₁₉H₂₄O₃, has been isolated from the normal gland, and has about one-fifth the potency of the testicular hormone, androsterone.

It can arise by oxidative replacement of the side-chain by =O in 11-dehydro-17-hydroxycorticosterone. A related androgen, *dehydro-iso-androsterone* occurs in traces in urine of both sexes, and, presumably, is formed in the cortex.

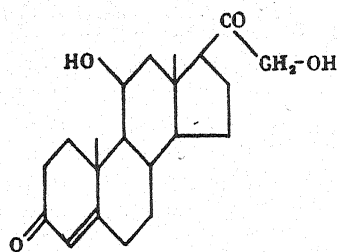
The adrenal cortex is very rich in steroids and in ascorbic acid, and is either a factory or a storage depot for these reactants. The metabolic disturbances due to cortical deficiency may be associated with lack of ascorbic acid, and there is evidence that this vitamin is of therapeutic value in the treatment of Addison's disease (Solare, 1937). The sexual disturbances associated with cortical dysfunction in the female originate probably from abnormal sterol metabolism, as a result of which the tissue either produces an androgen or fails to destroy one.

Ionic Action	Carbohydrate Action	
	Absent	Present
Retention	B	A C D
Na ⁺ + Cl ⁻		
Excretion		E F

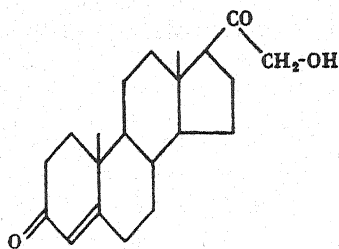
Metabolic Effects.



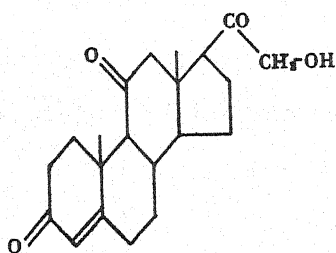
Cortical Hormone Pattern.



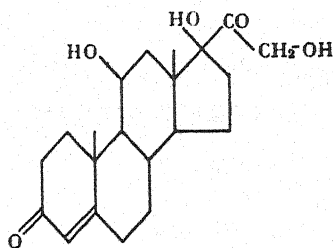
A, Corticosterone.



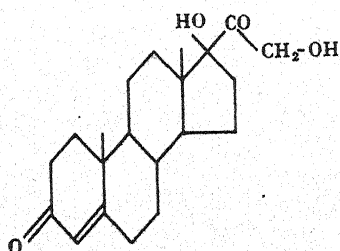
B, Desoxycorticosterone.



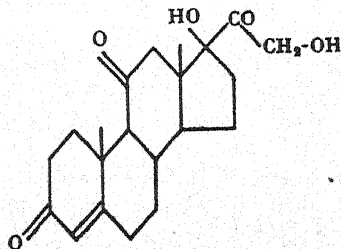
C, 11-Dehydrocorticosterone.



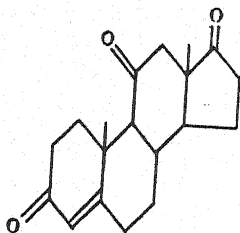
D, 17-Hydroxycorticosterone.



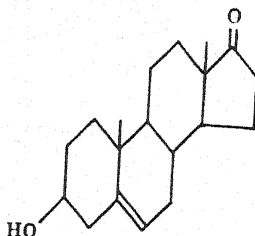
E, 17-Hydroxydesoxy-corticosterone.



F, 11-Dehydro-17-hydroxy-corticosterone.



G, Adrenosterone.



H, Dehydro-iso-Androsterone.

According to Thorn, *et al.* (*Science*, 94, 348, 1941), O : at 3, and a double-bond at 4 : 5, are essential for hormone activity in this series. All metabolic corticosterones have a $\text{—CO—CH}_2\text{OH}$ group at 17. HO— at 21 is necessary for carbohydrate action and Na-retention. HO— or O : at 11 decreases Na-retention, but augments carbohydrate action, which is further increased by an additional HO— at 17, although this induces Na-excretion. Hence, hormones E and F are very active in carbohydrate metabolism, but have no Na-retaining effect, in dogs ; while hormone B (desoxycorticosterone) has little action on carbohydrate metabolism, but is very effective in promoting Na-retention. Adrenosterone has no carbohydrate or Na-retaining effect. The output is often increased in malignant disease of the adrenal gland, and its detection in urine is of diagnostic value.

The therapeutic action of these and other steroid hormones can be prolonged by giving them in esterified form. Thus, in man, a daily injection of 10–15 mg. of desoxycorticosterone acetate, or *doca*, increases bodyweight and plasma volume, and reduces Na and Cl excretion in urine and sweat.

B. Adrenal Medulla

Adrenaline, or epinephrine, constitutes about 0.1 per cent. of fresh medullary tissue, and is present to a much lesser extent (0.02 per cent.) in the cortex. It is obtained from alcoholic or aqueous extracts of the gland, and is precipitable by ammonium hydroxide. The pure base is a crystalline solid, moderately soluble in water, and forming stable salts with strong acids. Structurally it is a catechol derivative of a methylated amino alcohol, and has been synthesised.

Effects of Adrenaline Injection.—Adrenaline is a sympathomimetic amine, and produces effects similar to the stimulation of structures innervated by the sympathetic system, the sweat-glands being a notable exception.

(1) *Vaso-constriction*.—Splanchnic and cutaneous arterioles are constricted, the arterioles of the skeletal muscles are unaffected or may be dilated. This leads to a redistribution of blood in the organism.

(2) *Cardiac Stimulation*.—The rate and force of the heart-beat are increased, the effect suggesting a heightened sensitivity to the Ca^{++} ion. The coronary arteries are dilated.

(3) *Glycogenolysis and Increase in Lactic Acid Content of Blood*.—This is accompanied by a corresponding breakdown of muscle glycogen. The excess lactic acid is resynthesised to glycogen in the liver, and in turn serves to raise the level of blood sugar. Thus, adrenaline injection causes a mobilisation of carbohydrate, and it may be described as a *glucotropic hormone*.

(4) Stimulation of creatine formation, as shown by rise in the creatine content of muscle.

(5) *Miscellaneous Muscular Responses*.—Dilatation of the pupil, retraction of the upper eyelid, and protrusion of the eyeball; relaxation of the bronchi, relaxation of the detrusor, and contraction of the sphincters in the bladder. Inhibition of movement, and contraction of the sphincters of the intestine.

In man, the therapeutic subcutaneous dose is 10 minims of a 0.1 per cent. solution, which represents about 0.003 mg., per kg. body weight.

Effects of Adrenaline Administration.—Injected subcutaneously the absorption of adrenaline is retarded owing to the local vaso-constriction it produces, and so it is administered intramuscularly or intravenously. The general cardio-vascular response depends on the integrity of the vagal innervation of the heart. If this be abolished, by section or by atropine, the response to adrenaline is a rapid, temporary rise in blood pressure, accompanied by an increase in the frequency and force of the heart beat. The effect subsides in a few minutes owing to destruction of the hormone. Where the vagal innervation is intact, moderate doses of adrenaline (0.1–0.5 mg.) cause a rise in blood pressure, accompanied by slowing of the heart, owing to vagal inhibition evoked by an aortic pressor reflex. Large doses of adrenaline (1.0–1.5 mg.) causes a 20 per cent. increase in the basal metabolic rate, and a two-fold increase in the cardiac output, the stimulus from the hormone being sufficient to overcome vagal inhibition. The cardiac effect may persist for over an hour, and may be accompanied by disturbances of rhythm, terminating in heart failure.

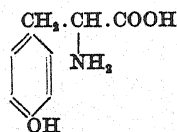
Significance of Adrenaline.—By means of cross-circulation experiments in which the blood of an intact animal was conveyed to the jugular vein of an adrenalectomised animal, Tournade and Heymans obtained evidence to show that adrenaline is continually being secreted in all conditions of bodily activity, reaching maximal values in (i.) physical stress, (ii.) exposure to cold, (iii.) fall in

arterial blood pressure, (iv.) asphyxia, (v.) hypoglycæmia, (vi.) central anæsthesia, (vii.) sudden or painful stimuli, and (viii.) emotional disturbance. Cannon for many years has maintained that adrenaline is the hormone concerned in the expression of terror, rage, panic and pain. By accepting the psychological implication of this theory, adrenaline appears as the chief effector agent employed by the unconscious self for the emergency defence of the organism.

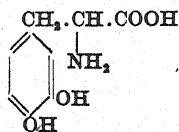
The adrenaline output is controlled by a higher nervous centre, which responds to (i.) stimuli from increase in blood pressure, these stimuli being transmitted from the aorta and carotid sinus, and serve in the maintenance of the circulation; (ii.) chemical stimuli from the sugar in the blood, which, when it falls below its usual level, evokes a compensatory release of stored carbohydrate; (iii.) hormone stimuli from the adrenotropic factor of the anterior pituitary; and (iv.) thermal stimuli, when the temperature of the external or internal environment falls below a critical value. In response to each of these types of stimuli, the adrenal gland sets in action the mechanism appropriate for meeting each specific demand. Emotional disturbances also may evoke liberation of adrenaline. The efferent impulses to the gland travel in the splanchnic nerves, and when these are cut the emergency secretion of adrenaline ceases.

Chemistry of Adrenaline.—In 1856, Vulpian observed that the adrenal medulla was coloured green by ferric chloride, and red by oxidisers such as iodine or chromate; and the term *cromaffin* was applied to the tissue. Extracts of the medulla showed similar chromogenic properties, which facilitated the isolation of the crystalline hormone, in 1901, by Takamine and by Aldrich, who showed that the molecular formula was $C_9H_{13}O_3N$.

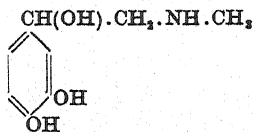
The green colour reaction with ferric salts indicated the presence of a catechol, or *o*-dihydroxy benzene nucleus, and this was confirmed by the production of protocatechuic acid (3:4-dihydroxy benzoic acid) on fusion of adrenaline with KOH. Von Fürth showed that a methylamino and a secondary alcohol group were present in the form of a side-chain, $—CH(OH).CH_2.NH.CH_3$, from which the carboxyl group of the protocatechuic acid was derived. Thus, the formula of adrenaline is 3:4-dihydroxyphenyl-ethanolmethylamine, and it is related both to tyrosine and to the tyrosine metabolite, dihydroxyphenyl alanine (dopa). Adrenaline has been synthesised independently by Stolz (1904) and by Dakin, starting from catechol. Like the other dihydroxy phenols, adrenaline is unstable in alkaline solution, and readily oxidised to a red pigment.



Tyrosine.



"Dopa."



Adrenaline.

Assay of Adrenaline.—The hormone value of preparations may be estimated (i.) *colorimetrically*, by comparison of the blue colour it yields with Folin's phosphotungstate reagent; (ii.) *biologically*, from the inhibitory effect on the contraction of a strip of involuntary muscle suspended in saline, the accelerating effect on an isolated heart, the dilating effect on a denervated iris, and the vaso-pressor response in an animal.

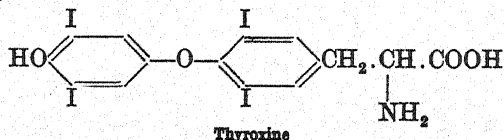
Adrenaline is optically active owing to an asymmetric carbon atom in the side-chain. The natural form is the (–)-isomer, and is about eighteen times as potent as the (+)-isomer.

THYROID HORMONE

The thyroid gland arises as an outgrowth of the pharyngeal floor in the embryo. In the human adult it weighs 20–25 gm., and is unique among the tissues because of its high content of iodine, the average amount being 15 mg., at least half of which can be extracted in the form of the hormone thyroxine.

L-Thyroxine was isolated by Kendall, in 1914, and identified by Harington and Barger, who subsequently effected its synthesis (1927). The details of this achievement are summarised by Barger (1930).

Thyroxine is derived from two molecules of di-iodo-phenol, and contains an alanine side-chain, thus being related to tyrosine, its precursor in the diet, and to iodo-gorgic acid. Owing to the asymmetric carbon atom in the side-chain, two forms of thyroxine are known, the natural hormone being the L (–)-isomer, derived from L-tyrosine.



Thyroxine

There is reason to believe that the actual hormone is a compound of thyroxine, since preparations of gland protein have been obtained that are more effective than pure thyroxine, and act more rapidly. Also, thyroxine has been isolated from the thyroid as a globulin component. The residual iodine of the gland is present as the physiologically inert di-iodo-tyrosine.

Effects of Thyroid and Thyroxine Administration.—Thyroid preparations are exceptional among the autacoids in that they are effective when administered by the mouth, from which it is concluded that the gland originally sent its secretion into the primitive alimentary tract.

(1) *Stimulation of Metabolism.*—One mg. of thyroxine can raise the basal metabolic rate of the adult by 2–3 per cent. Larger doses cause a proportionate increase and a prolonged effect. This stimulation is accompanied by a loss in weight, owing to depletion of the fat reserves, and hypertrophy of the active organs, heart, kidney, suprarenal, and liver.

(2) *Mobilisation of glucose*, owing to increased breakdown of hepatic and cardiac glycogen. The blood sugar level is raised, and hyperglycæmia and glycosuria may result.

(3) *Decalcification of bone.* The effect differs from that of the parathyroid hormone in that the plasma calcium level is not raised and the transferred calcium is excreted by the intestine as well as in the urine.

(4) *Stimulation of metamorphosis* in amphibia, including the tadpole.

(5) *Species desensitisation to methyl cyanide* (aceto-nitrile). A method for assaying thyroid preparations is based on the increased tolerance to methyl cyanide that they confer on mice.

Hypothyroidism.—A. *Acute.*—Thyroidectomy, uncomplicated by involvement of the parathyroids, in young animals causes cretinoid conditions to develop, including retardation of skeletal growth, hardening of skin and loss of hair, retarded sexual development and absence of secondary sexual characters, and mental dullness and apathy. In man, the condition of *cachexia strumipriva* (κακός, ill; ἔξις, habit; *struma*, goitre; *privus*, deprivation), or operative myxœdema, develops in a week, as shown by: fall in basal metabolic rate, usually by about 25 per cent.; slowing of the heart beat to about 50 p.min.; subnormal temperature; cutaneous changes, loss of hair from scalp and outer third of eyebrows, subcutaneous deposition of myxomatous tissue; disturbance of mental activity and sexual functions.

B. *Chronic.*—Non-development or atrophy of the gland.

(1) Congenital maldevelopment causes **cretinism**, the signs of which do not appear until about six months after birth, due partly to the autacoids supplied in the mother's milk and partly to the reserve in the child. The signs and symptoms of cretinism are similar to those of hypothyroidism or myxœdema.

(2) Simple goitre accompanied by hypothyroidism may arise when there is a deficiency of iodine in the diet. This is due usually to a faulty environment. A compensational hypertrophy

of the gland endeavours unsuccessfully to manufacture sufficient hormone from inadequate material.

(3) Atrophy of the thyroid may occur spontaneously in adults, especially women, after middle age.

All these conditions are specifically relieved by appropriate thyroid administration. One mg. of iodine *per diem*, supplied in any inorganic form, is sufficient to cure simple goitre and prevent its reappearance.

Hyperthyroidism.—Increased secretion of the hormone may occur without obvious signs of gland enlargement, the result being : (i.) increased basal metabolism, (ii.) mild pyrexia, (iii.) pigmentation of the skin, (iv.) nervous restlessness, (v.) mild exophthalmos, (vi.) cardiac and vascular disturbance.

Hyperthyroidism is a feature of Graves's disease (Basedow's disease, or exophthalmic goitre), the pathology of which is still obscure. In Graves's disease the basal metabolic rate may be 50 per cent. above normal in an average case, and up to 100 per cent. above normal in a severe case.

Metathyroidism.—Production of abnormal secretions of the gland may complicate many varieties of Graves's disease and toxic adenomata of the gland. The condition is sometimes termed "thyrotoxicosis," "toxic goitre," and "dythyroidism." The chief symptoms are due to hypersensitivity of the sympathetic system. Metathyroidism is distinguished from hyperthyroidisms by being benefited by iodine administration, which suggests that it is due to liberation of an immature or defective autacoid. Harington, however, can find no evidence of an abnormal hormone.

The most obvious histological effect of the iodine therapy is the restoration of the colloidal appearance to the gland structure.

Regulation of Thyroxine.—The thyroid gland is supplied by the middle cervical sympathetic ganglion, but section of the entire nerve supply does not lead to hypothyroid states, from which it is inferred that the gland has a metabolic activity of its own, and continually elaborates, stores and secretes its hormone independently of the autonomic nervous system. Apart from the obvious limiting factors, namely, the supply of iodine and tyrosine or phenyl alanine, the gland responds to (i.) hormone control by the thyrotropic factor of the anterior pituitary, and (ii.) thermal stimuli, which, perhaps, act through the thermotaxic centre in the hypothalamus, and reach the gland by the sympathetic nerves.

Cramer has shown that exposure to cold evokes a protective secretion by the thyroid as well as by the adrenal gland.

Therapeutic Applications of Thyroxine.—In addition to their specific use in the hypothyroid states, thyroid preparations are used

popularly in the treatment of obesity. Given to a normal subject, small doses lead to a disappearance of storage lipides, provided the diet is kept sufficiently low in fat and carbohydrate to compel the subject to draw on his own reserves. Overdosage leads to alimentary disturbances, tachycardia and loss in weight. Other drugs, notably di-nitro phenol and di-nitro *o*-cresol, have a similar effect in increasing general metabolism, but they are dangerous remedies in comparison with the natural hormone. Unlike thyroxine, they display a catalytic effect in promoting respiration in tissue slices (Dodds).

Significance of Thyroxine.—The iodine content of the thyroid gland varies greatly with the environment and the diet. The dried preparations of ox, sheep or pig gland, used therapeutically, are standardised by admixture with sufficient lactose to give a powder containing 0.1 per cent. iodine in the form of thyroxine. The iodine content of human and other animal thyroids ranges from 0.1 to 0.5 per cent. of the dry weight, but may exceed 1.0 per cent. in animals living on marine foodstuffs rich in the halogen, such as Orkney sheep, which feed on kelp seaweed. When the iodine content falls below 0.1 per cent. of dry weight, the gland enlarges and assumes the goitrous form.

Thyroxine has only about half the activity of a thyroid preparation containing the same amount of iodine, from which it appears either that another and much more potent autacoid is present, or that thyroxine is merely a constituent of the actual autacoid.

Furthermore, thyroxine differs from adrenaline in that the response to its administration is very slow and sustained. Adrenaline reacts within a minute; thyroxine requires two to four days. This suggests that thyroxine requires physiological modification before becoming available for metabolic catalysis.

The daily output of thyroxine in the human adult is believed to be of the order of 0.5–1.0 mg. *per diem*, which is the amount sufficient to maintain normal health in the myxoedematous subject.

The iodine thus metabolised is not lost to the organism, but is in part re-synthesised to thyroxine. The urinary output of iodine ranges from about 0.03–0.173 mg. *per diem*, depending on the diet, and von Fellenberg has computed that the minimal requirement of the human subject is met by the daily provision of 0.05 mg. I_2 in any soluble inorganic form.

Using radioactive iodine, Chaikoff has found that it reaches the thyroid within a few minutes after alimentary absorption, and in two hours can be detected in the actual hormone.

Artificial metabolic stimulants, such as di-nitrophenol, are also effective in raising the metabolic rate to the normal level when

administered to myxœdematous subjects, but fail to benefit the other associated disturbances, which is additional proof that the thyroid gland controls other processes in addition to its calorogenic action.

Thyroxine added to tissue preparations has little or no effect on tissue respiration, though it is claimed that tissues from animals suffering from hyperthyroidism display an increase in metabolic activity, including glycolysis and lactate formation.

Thyroid Inhibitors.—The purine, *Paraxanthine*, and some thiols, including *thiourea*, can inhibit the activity of the thyroid. *Paraxanthine* is a normal microconstituent of urine. Volatile thiols are obtainable from *brassicæ*, including cauliflower and cabbage, and may be responsible for their goitrogenic effect, recorded by McCarrison.

PARATHYROID HORMONE

The parathyroids are the smallest endocrine organs known in the animal body, and are represented by four structures situated at the back of the thyroid gland. Before their existence was recognised, they were removed with the thyroid tissue during experimental thyroidectomy, and the effects of the operation included both hypothyroid and hypoparathyroid phenomena.

Parathyrin, or parathormone (Collip), is obtained by extracting fresh parathyroid tissue with 5 per cent. HCl, and precipitating the higher proteins. Parathyrin is a proteose, and is destroyed by the proteases of the alimentary tract. Subcutaneous or intravenous injection causes :—

(1) *Mobilisation of Blood Calcium.*—The Ca level is raised from the normal value of about 10 mg. per 100 ml. to a value depending on the number of units given.

The unit is one-hundredth of the amount required to cause a 5 mg. Ca increase per 100 ml. in the blood serum of a 20 kg. dog, fifteen hours after injection.

(2) *Abolition of Tetany.*—All forms of tetany due to hypocalcæmia are relieved, including those directly due to parathyroid removal. This includes infantile, post-operative, and parathyroid tetany, as well as that due to alkalosis.

(3) *Hypophosphatæmia.*—The rise in blood calcium is associated with a fall in blood phosphate, and an increase in urinary phosphate output.

Hyperparathyroidism.—A. *Acute.*—Collip has shown that overdosage of parathyrin causes severe hypercalcæmia in which the Ca level may rise above 20 mg. per 100 ml. This is accompanied by (i.) depression of motor excitability ; (ii.) anorexia, drowsiness, and

coma; (iii.) diarrhoea; (iv.) decrease in blood volume and increase in viscosity.

Death is due to failure of the heart, and is followed rapidly by intravascular clotting.

B. Chronic.—Clinical hyperparathyroidism has been recognised by Barr and other workers. The features are: (i.) mobilisation of calcium from the skeleton; (ii.) bone rarefaction; (iii.) hypercalcaemia; (iv.) negative calcium balance with hyperexcretion of calcium in the urine; (v.) muscular weakness and hypotonia.

Hypoparathyroidism.—**A. Acute.**—Complete parathyroidectomy in the dog is followed by: (i.) hypocalcaemia, the serum Ca level falling by 50 per cent. to about 6 mg. per 100 ml.; (ii.) hyperexcitability of the peripheral nerves to electrical and mechanical stimuli, muscular twitching; (iii.) tetanic spasm, due to central stimulation in the brain stem. These increase in violence, with alternate phases of exhaustion, until death results from asphyxia owing to respiratory spasm, or cardiac failure.

Acute hypocalcaemia may be treated by oral administration of large doses of soluble calcium salts, by saline injections, and, specifically, by injections of parathyrin. After about six weeks the organism becomes adapted to life at a lower level of serum calcium.

B. Chronic.—Idiopathic hypoparathyroidism and some forms of infantilism exemplify the chronic condition. Infantile tetany, which is associated with rickets, coeliac disease and steatorrhoea, is not regarded as being a parathyroid disorder.

Hypoparathyroidism, as far as it can be regarded as a clinical entity, displays: (i.) hypocalcaemia; (ii.) hyperphosphataemia; (iii.) actual or potential tetany. Probably the age of onset of the disease determines the nature of the symptoms.

Calcium-mobilising steroids, including vitamin D₂ and dihydro-tachysterol (AT10), are used therapeutically in treatment of parathyroid deficiency.

Significance of Parathyrin.—The hormone regulates the renal excretion of PO₄, and thus controls the PO₄ level, and, indirectly, the Ca level in the plasma. Parathyrin secretion or injection stimulates excretion of urinary PO₄. To compensate for the resulting hypophosphataemia, PO₄ is withdrawn from the bones, with accompanying release of Ca, which leads to a hypercalcaemia.

The artificially prepared steroid, dihydro-tachysterol (AT10, or anti-tetanic substance 10) has a similar effect, but can be given by mouth.

Tetany.—Clinical tetany is characterised by the tendency of muscles to undergo spasmodic contraction, those commonly affected being the muscles of the forearm and hands, the larynx, and, less

frequently, the lower limbs. The condition is associated with a fall in the plasma calcium level below 7 mg. per 100 ml., and an accompanying rise in the phosphate level to above 5 mg. per 100 ml. Tetany is recognised clinically by :

(i.) *The Chvostek Sign.*—Twitching of the upper lip and side of the face elicited by tapping the facial nerve in front of the ear.

(ii.) *The Trousseau Sign.*—Carpal spasm elicited by pressure on the upper arm.

(iii.) *Laryngismus Stridulus.*—Closure of the glottis owing to sudden spasm of the laryngeal muscles. Inspiration ceases until the muscles relax, when the air re-enters the lungs with a characteristic sound.

Latent tetany can be recognised by the increased excitability of the motor nerves to a constant, low voltage electrical stimulus; the cathodal opening response being evoked by a current of 0.6–3 milliamperes, the normal requirement being about 6.0 milliamperes.

PANCREATIC HORMONES

History.—In 1889, Mehring and Minkowski found that extirpation of the pancreas in dogs was followed by severe glycosuria and fatal coma, the condition being indistinguishable from diabetes mellitus. Ligation of the gland duct, although causing digestive disturbances from lack of pancreatic juice, did not evoke this artificial diabetes, from which it was concluded that a hormone or internal secretion was still being manufactured.

In 1909, De Meyer suggested that the hormone was made in the insular tissue of the gland, and Schafer suggested the name *insuline*. Attempts to extract this hormone had been inconclusive, although Zuelzer (1907) and Scott (1911) undoubtedly obtained active preparations, until, in 1922, Banting and Best devised a technique for separating the autacoid from the destructive influence of the pancreatic enzymes.

By continuous injection of islet extracts, a depancreatized dog was kept alive for seventy days in 1922. Less than two years later, insulin was in use all over the world in the successful treatment of diabetes.

Preparation of Insulin.—(a) *Alcohol Fractionation* (Collip, 1923).—Fresh minced pancreas is extracted with aqueous alcohol, the extract is concentrated *in vacuo* until most of the fat has separated out, alcohol is added to precipitate the tissue proteins, and then insulin in the filtrate is precipitated by raising the alcohol concentration to 92 per cent., or by addition of ammonium sulphate. It is purified by reprecipitation at the iso-electric point, pH 5.3.

(b) *Picric Acid Precipitation* (Dickens and Dodds, 1924).—The fresh gland is ground up with solid picric acid, extracted with acetone, and the insulin picrate recovered after distillation of the solvent.

Crystalline insulin has been obtained by various methods, including the addition of pyridine to insulin solutions at pH 5.6. The potency of the crystalline hormone is very little greater than that of some of the amorphous preparations, namely 23–26 international units per mg. Chemically, insulin belongs to the albumins, which explains its destruction in the alimentary tract, when given by the mouth.

Effects of Insulin Injection.—(1) *Hypoglycæmia*.—The level of the blood sugar falls rapidly within thirty minutes of the injection; and, if the dose be sufficiently large, and the carbohydrate reserves depleted by starvation, the condition of hypoglycæmia sets in, the blood sugar level having fallen below 0.04 per cent.

The condition in the rabbit is marked by signs of extreme hunger, violent convulsions, coma, and death from respiratory failure. Rigor mortis follows immediately. In the human subject, hypoglycæmia is accompanied by hunger pangs, exhaustion, tremor, vaso-motor disturbances, sweating, delirium, and coma.

All these hypoglycæmic phenomena may be abolished in a few minutes by intravenous injection of glucose, or less rapidly, by oral administration. Fructose and mannose are not so effective; galactose is almost without action. Injection of the other glucotropic autacoids, namely, adrenaline and pituitrin, may cause a rise in blood sugar sufficient to compensate for the effects of the insulin.

Insulin injection is followed by an increased metabolism and a rise in the respiratory quotient, showing that the utilisation of sugar has been stimulated.

The hypoglycæmia, however, is greater than can be accounted for by the immediate combustion of glucose, and two other factors are believed to act in lowering the blood sugar level: (i.) increased storage of carbohydrate, and (ii.) decreased sugar formation from the glucogenic amino acids. Accompanying the rise in carbohydrate metabolism, is an immediate inhibition of ketone formation from fat metabolism.

(2) *Glycogenesis*.—When a diabetic animal is given insulin and more than sufficient sugar to compensate for the insulin hypoglycæmia, it is found that some of the excess of the saccharide has been stored up in the liver and muscles as glycogen. This glycogenic effect is not seen usually after insulin injection because the glycogen stores are rapidly depleted in an endeavour to maintain a normal sugar level in the blood.

Muscle glycogen represents a much more stable location of the polymer than hepatic glycogen. Severe hypoglycæmia ultimately results in an almost complete withdrawal of liver glycogen to meet the aggravated metabolic demands, whereas even a fatal hypoglycæmia may leave an abundant reserve of muscle glycogen.

(3) *Hypophosphatæmia* follows insulin injection, probably owing to a diversion of serum phosphate to form labile hexose phosphate preparatory to sugar utilisation.

The general effects of insulin are expressed by a hypoglycæmia attributable to (i.) increased sugar oxidation, (ii.) increased sugar storage, chiefly in the muscles, and (iii.) decreased sugar formation from non-carbohydrate sources.

The Insulin Unit.—This was defined originally as the amount required to evoke hypoglycæmic convulsions in a fasting rabbit of 2 kg. weight. Wide variations in animal sensitivity have led to the abandonment of this unit in favour of one based on the use of a standard preparation of crystalline zinc insulin, 1 mg. of which represents 22 units. One insulin unit equals 0.045 mg. of this material.

Insulin forms a salt with protamines and with globin that is more prolonged in action than the free hormone, and this therapeutic effect may be augmented by zinc, which is present in 0.5 per cent. concentration in crystallised insulin.

Regulation of Output.—The hormone is continually being secreted to meet demands of carbohydrate metabolism, and is present in the venous blood leaving the pancreas. Secretion is evoked (i.) *chemically*, by a rise in the sugar content of the blood, and (ii.) *neurally*, by the vagi which innervate the insular tissue, and which on stimulation bring about a fall in blood sugar. The sugar factor in the secretion of insulin has led some workers to attribute diabetes mellitus to failure of the gland owing to prolonged exhaustion by a diet excessively rich in carbohydrate. This notion in a less explicit form inspired the earlier methods of treatment of the disease by complete exclusion of all starches and sugars from the diet, so that the gland might recuperate. The discovery of insulin, however, has made it possible to utilise carbohydrate foodstuffs, and many types of dietary are now available for the diabetic subject. The occasional appearance of an insulin-resistant type of diabetes is attributed to dysfunction involving other gluco-kinetic hormones, notably those of the anterior pituitary.

Significance of Insulin.—The hormone is a crystallisable albumin of m.w. 35,000, and contains 12 per cent. of tyrosine, the hydroxyl group of which is necessary for the activity of the hormone, as shown when it is masked by acetylation. Insulin promotes utiliza-

tion of pyruvate in respiring muscle (Rice and Evans, 1939), and phosphate exchange by *ATP* (Sacks, 1943), but up to the present time no glycolytic property has been ascribed to insulin when mixed with glucose in aqueous solutions or when added directly to blood. The existence of a non-pancreatic factor was established when Houssay, Lucke and other workers (1931-37) showed that removal of the pituitary gland from pancreatectomised animals abolished both glycosuria and ketonuria, thus disproving the theory that diabetes is always of purely pancreatic origin. This effect of the pituitary is provisionally explained by endowing it with two hormones; (i.) a *diabetogenic* factor, which antagonises insulin, and, if present in excess, leads to a hyperglycæmia of the insulin-resistant type, and (ii.) a *ketogenic* factor, which promotes the degradation of fatty acids, and, possibly, their transformation into glucose or glycogen.

GYNÆCOGENS: FEMALE SEX HORMONES

In order that the genetically determined vertebrate sex may develop to maturity and function, hormones elaborated by the germinal glands are necessary. These are sometimes termed the secondary sexual hormones to distinguish them from the primary sexual autacoids responsible for the sex of the embryo, which at the present time is beyond the scope of experimental control. The various aphrodisiacs employed by the human race have a story that goes back to the fruit tree of Eden, and includes the fantastic pharmacologies of the East as well as the potions of the Middle Ages. It is now established that the sex hormones, both male and female, are lipid compounds related to the sterols, and are only absorbed with difficulty from the alimentary tract, which may explain the erratic results obtained by empirical therapy.

The experimental foundation of modern knowledge dates from 1912, when Nussbaum showed that the sex characters of the male frog are controlled by a testicular hormone, and that whenever specific structures are associated with animal sex their growth is dependent on chemical factors. Because of the greater complexity of the female organism, two types of hormone are required: (i.) an *œstrogen*, controlling the early stage in the uterine cycle, and (ii.) a *progesterone*, which prepares the uterus for the implantation of the fertilised ovum.

Progress in the study of the human hormones was delayed, partly through ignorance of the exact nature of the menstrual cycle, and partly because of the lack of a satisfactory test for measuring the activity of the hormone preparations. In 1923, Allen and Doisy

obtained a lipid extract from ovaries which, when injected into castrated rodents, was able to re-establish the reproductive or œstrus cycle, and obviously contained the long-sought œstrogen. The same workers also adapted a "vaginal-smear" test, whereby it was possible histologically to detect the onset of œstrus from changes in the vaginal epithelium.

In 1927, Aschheim and Zondek found that an œstrogen was excreted in large quantities in the urine of pregnant animals, thus providing a source of material for purification and identification. Since then, five closely related œstrogens have been isolated from the ovary, the urine of pregnancy, and from the placenta; a progesterone has been isolated from the corpus luteum, and four androgens, or male sex hormones have been isolated from various sources. All are derived from a parent *cyclo-pentano phenanthrene* nucleus (p. 188).

Œstrogens.—The ovarian follicular hormone, made in the ripening follicle, is a typical œstrogen, an evoker of sex response in the female. Œstrogens prepare the uterine lining for reception of the fertilised ovum, and stimulate the growth of the mammary glands. At birth time, they activate the uterus. They are used therapeutically to lessen the effects of the sudden onset of the menopause.

The first œstrogen isolated was *œstrone*, or *theelin*, which was got in crystalline form from urine by Doisy (1929). It is made in the ovaries and in the placenta. In 1935, Doisy obtained a dihydro-theelin, *œstradiol*, from ovarian tissue, in a yield of 6 mg. per ton of sow ovary, which indicates the low concentration and corresponding high potency of the hormone. Œstrone was synthesised in 1940 by Bachman.

Gonadotropins.—Sex glands do not function independently, but are controlled by gonadotropic factors from the anterior pituitary. Good sources of these are human urines during pregnancy, or after ovariectomy, or after menopause. These gonadotropins have been resolved into a follicle-stimulating hormone (FSH) and a luteinising hormone (LH), but their identification is not yet complete. All species of animals do not respond alike to gonadotropin administration (Doisy and Smith, 1940).

Œstradiol is the actual ovarian follicular hormone. It is a colourless steroid, slightly soluble in water, but freely soluble in organic solvents. Chemically, it is the 3:4-dihydroxy derivative of *œstrane*, or 13-methyl *cyclo-pentano phenanthrene*, the monomethylated steroid nucleus that occurs in all the natural œstrogens (p. 199). *Œstrone* and *œstriol* represent various oxidation forms in which œstradiol is excreted, after esterification, in the urine. The most active of these compounds is œstradiol, and the least

The Natural Oestrogens

Name.	Formula.	Sources.	Discoverer.	Relative Potency of 0.001 mg. (1 γ).
(1) Oestradiol	$C_{18}H_{24}O_2$	Ovary.	Doisy, 1935.	20 m.u.
(2) Oestrone (Theelin)	$C_{18}H_{22}O_2$	Ovary. Urine.	Doisy, 1929. Butenandt, 1929.	10 m.u. (= standard)
(3) Oestriol (Theelol)	$C_{18}H_{24}O_3$	Palm kernel. Placenta. Urine. Willow tree.	Dingemans, 1929. Collip, 1930. Marrian, 1930.	0.1 m.u.
(4) Equilin	$C_{18}H_{20}O_2$	Urine.	Girard, 1930.	1.0 (?)
(5) Equilenin	$C_{18}H_{18}O_2$	Urine.	Girard, 1930.	0.5 (?)

active is oestriol, which on account of its three hydroxyl groups is more soluble in water and less soluble in fat-solvents than the other oestrogens. Oestriol is manufactured also in the placenta, during pregnancy, and is excreted partly as a glucuronic acid derivative.

Physiological Effects of Oestrogens.—In experimental animals, whose ovaries have been removed, injection of an oestrogen causes rapid growth of the vaginal epithelium, as in normal oestrous states, together with increased growth of the uterine mucosa and the mammary tissue. In the normal animal, oestrogen injection hastens the onset of oestrus. These effects are accompanied by (i.) increased metabolism, with loss in weight, especially in obese gonadectomised animals; (ii.) glucose mobilisation and increased resistance to insulin.

Excessive doses of natural or artificial oestrogens inhibit the gonadotropic activity of the pituitary gland (Noble, 1938).

Standardisation of the Oestrogens.—The original standard was the rat unit (r.u.) or the mouse unit (m.u.), which was defined as the minimal quantity of hormone necessary to evoke oestrus in the gonadectomised animal, the state being recognised histologically by the Allen and Doisy test. These units are apt to vary according to differences in laboratory technique, and a committee established by the League of Nations has recommended an international unit equivalent to 0.1 γ (0.0001 mg.) of oestradiol benzoate, which is the average value of the mouse unit.

The Luteal Hormone

The corpus luteum, which is formed from the ovarian follicle after escape of the ovum, elaborates a hormone, *progesterone*, or *progestin*, that in the human non-pregnant condition acts by stimulating the growth and secretion of the endometrial mucosa during the fourteen days prior to menstruation. During pregnancy, the corpus luteum

persists, maintained by an autacoid secreted by the embryo, and the output of progesterone continues in most animals, except the mare, until parturition. Thus, ovarian activity is associated with the production of two independent hormones, oestradiol and progesterone.

Progesterone, or *luteosterone*, $C_{21}H_{30}O_2$, the luteal hormone, was recognised by Corner, Allen, Gley and other independent workers, and obtained in pure crystalline form by Butenandt. It is a methoxy-keto derivative of a dimethyl steroid nucleus similar to that found in the androgens, and is responsible for the three principal progestational functions of the corpus luteum :—

(1) Premenstrual endometrial growth and secretion, in the non-pregnant state.

(2) Inhibition of ovulation and menstruation during pregnancy.

(3) Embedding of the fertilised ovum, and placenta formation.

Progesterone is excreted as its inactive reduction derivatives, *allo-pregnanediol* and *pregnanediol*, which occurs as a glucuronide in human urine during the latter half of the menstrual cycle, the daily yield being 1–10 mg., as the Na salt. Progesterone has been obtained, by Marrian, from pregnanediol and from the plant steroid, *stigmasterol*.

Natural sources, such as the ovary of the sow, yield only about 3 mg. pure progesterone per 100 kgm. of material, representing 25 kgm. of corpora lutea. The hormone is used therapeutically in treatment of uterine disorders, menorrhagia, threatened abortion and spasmodic dysmenorrhœa. A synthetic steroid, *pregneninolone*, has the advantage that, unlike pregnanediol, it is active when given orally.

The international unit (1935) is defined as the potency of 1 mg. of crystalline progesterone.

Significance of the Ovarian Hormones.—Extirpation of the ovary before puberty inhibits the development of the secondary sexual characters and the establishment of the menstrual cycle. Extirpation after puberty induces the syndrome characteristic of the menopause, or climacteric, which in the human subject normally occurs between the ages of forty-five and fifty, and is often associated with a general endocrine disturbance. Ovarian grafts in the ovariectomised subject lead to a temporary restoration of the sexual cycle, but the implanted tissue degenerates usually within a year. Ovarian grafts in the normal male animal are unsuccessful owing to the antagonising effect of a testicular factor. If, however, the animal is previously castrated, the ovarian graft displays a feminising effect, as shown by growth of the mammary glands and partial assumption of the secondary female sex characters. Oestrogens repeatedly injected into normal male animals evoke

prostatic hypertrophy and enlargement of the utriculus, which is the homologue of part of the genital tract in the female.

On account of the importance of the ovary, it was formerly assumed that the tissue was more or less autonomous, and produced the hormones automatically; but it is now known that ovarian activity is completely subservient to the control of the anterior pituitary gland, and that many conditions ascribed to ovarian incompetence are due to pituitary dysfunction (Parkes, 1930).

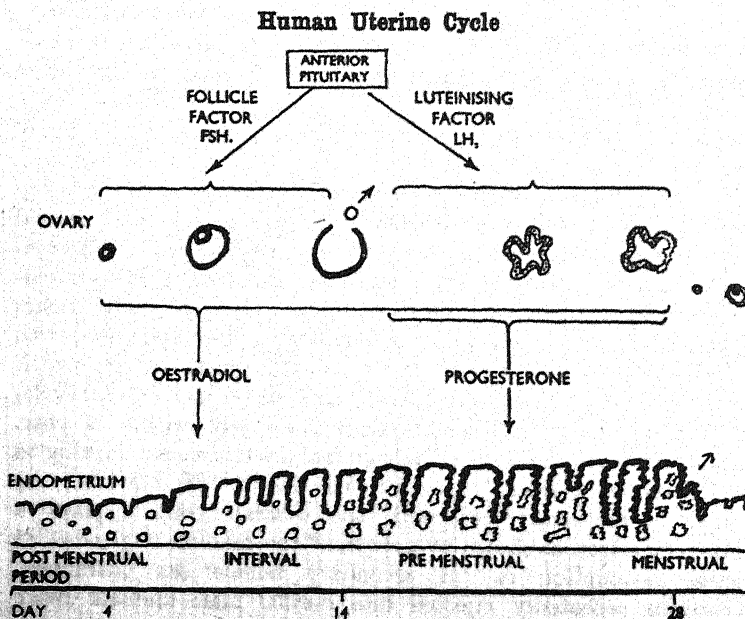
Pituitary Factors in Ovarian Activity.—The anterior lobe of the pituitary gland secretes two factors, which on account of their action on the sex glands are called *gonadotropic hormones*.

(1) *Follicle-stimulating Hormone* (FSH), "prolan A," or factor A, promotes development of the Graffian follicle in the ovary.

(2) *Interstitial-cell-stimulating Hormone* (ICSH), *Luteinising Hormone* (LH), "prolan B," or factor B, brings about formation of the corpus luteum. FSH and LH are glyco-proteins, FSH occurs chiefly in acid extracts of the anterior pituitary, and LH in alkaline extracts.

Rupture of the follicle and liberation of the ovum is due to rise in pressure within the follicle, following increased secretion of oestradiol.

Thus, it appears that the uterine cycle in non-pregnant and in pregnant conditions is controlled by the operation of two distinct groups of hormones, as shown in the following diagram.



The Menstrual Cycle

Days.

- 1-4 Secretion of FSH induces secretion of oestradiol by granulosa cells of the follicle.
- 5-11 Rapid growth of follicle. Continued secretion of FSH and oestradiol. Regeneration of the endometrium.
- 12-14 Rupture of follicle and escape of ovum. Secretion of LH. Formation of the corpus luteum.
- 15-26 Continued secretion of LH. Persistence of corpus luteum. Secretion of progesterone. Enlargement and secretory activity of endometrial glands.
- 27 Inhibition of pituitary hormones by the increase in the output of oestradiol. Corpus luteum starts to degenerate.
- 28 Onset of menstruation. Cessation of secretion of FSH. Rapid degeneration of corpus luteum. Decline in secretion of progesterone and oestradiol.
- 1-4 Decrease in secretion of ovarian hormones leads to renewal of pituitary activity, and continuation of the cycle.

Hormones in the Urine of Pregnancy.—During pregnancy, the persistence of the corpus luteum is assured by the continued secretion of LH, with the result that three types of hormones appear in the urine: (i.) Oestrogens, which are excreted in the inactive esterified form, so as to protect the foetus from the powerful hormones of the mother; (ii.) progesterone, excreted in the inactive form of pregnanediol; (iii.) gonadotropic hormones.

In human pregnancy urine, oestrone and pregnanediol occur; in urine of the cow or mare, chiefly oestriol. Both oestrogens are excreted as inactive glycuronic derivatives, until the onset of labour, when they appear in the free form (Cohen and Marrian, 1935).

Oestrogens and Pituitary Gonadotropic Hormones in Urine (Representative values, expressed in mouse units)

	Oestrogens.				Gonadotropins, per Litre.	
	Non-pregnant.		Pregnant.			
	Per Litre.	Per Diem.	Per Litre.	Per Diem.	FSH.	LH.
Woman .	425	600	21,000	31,000	20,000	10,000
Man .	160	240	—	—	—	—
Mare .	200	2,000	100,000	1,000,000	300	0
Stallion .	170,000	1,700,000	—	—	—	—
Bull .	330	—	—	—	—	—

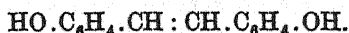
Small quantities of oestrogen appear in human urine about the fifteenth day of the menstrual cycle, and disappear just before the onset of menstruation. During pregnancy, the output rapidly increases to a maximum of 1.5-2 mg. per litre, which persists till parturition, and then rapidly falls to the normal minimum.

The oestrogens presumably arise in the developing placenta, as, during pregnancy, the ovarian oestrogens are suppressed, and no follicles are ripening.

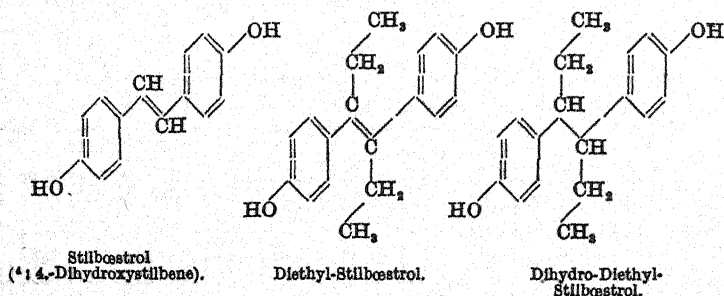
Equilin and equilenin occur as reduction derivatives, along with oestrone in the pregnancy urine from the mare, which has a total oestrogen value of about 10 mg. per litre, or five times as great as that of human pregnancy urine. Stallions differ from other male animals in excreting a urine very rich in oestrogens, the hormones having been formed in the testicle, along with the androgens.

The gonadotropin of pregnancy urine also occurs in the blood and the placenta. It is a *chorionic gonadotropin*, made in the chorionic tissue, and somewhat resembles the luteinising hormone of the anterior pituitary.

Non-steroid Oestrogens.—Dodds and other workers have prepared a number of cyclic compounds capable of evoking oestrus, the most potent being derivatives of 4:4'-dihydroxystilbene, a parent hydrocarbon which Dodds terms "stilboestrol," $C_{14}H_{12}O_2$.



This discovery shows that the steroid ring is not necessary for oestrogenic function. *Diethyl stilboestrol* is about two and a half times more potent than oestrone, while the more powerful *dihydro-diethyl-stilboestrol* is as active as oestradiol.



Oestrogenic activity requires the presence of the phenolic $-OH$, when this is esterified as acetate or propionate, the drug is only slowly absorbed and as such may be implanted in subcutaneous tissue, where it hydrolyses. The stilboestrols have the advantage

over the steroid oestrogens in being more soluble and capable of being absorbed when given orally, and also in their lower cost of production. Given in prolonged doses, they, like the natural oestrogens, repress the gonadotropic effect of the anterior pituitary, and, thus, indirectly can cause the regression of prostatic carcinomata due to stimulation by androgens.

Sources of the Oestrogens.—Estradiol and oestrone can be obtained from ovarian tissue of domestic animals, and display no species specificity. That these or other oestrogens can arise independently of the follicle is shown by the fact that the oestrus cycle can persist after complete destruction of the ovarian follicles by short-wave irradiation, and also by the persistence of oestrogens in urine after ovariectomy. The placenta and the adrenal cortex have been suggested as extra-ovarian sources of the hormones. Commercially, oestrogens are now obtained in quantity from the urine of the cow or the horse, an unexpected discovery being that the female sex hormones are excreted both in male and in female urine, and, conversely, the male sex hormones have been isolated from female urine. This paradox is explained by the close chemical relationship between both groups of compounds, each member of which is derived from a methylated steroid nucleus. Vegetable sources are represented by palm kernels and palm oil.

Biological Tests for Pregnancy.—The detection of the chorionic gonadotropic factors in urine constitutes a very delicate and trustworthy test for pregnancy. The test depends on the injection of the urine into young mice (Aschheim-Zondek test), rabbits (Friedman test), or a species of toad, *Xenopus laevis* (Hogben test), and the observation of subsequent histological changes.

Aschheim-Zondek Test.—Five mice, aged three to four weeks, and weighing 6–8 gm. each, are injected individually with 1.2–2.4 ml. of urine, divided into six doses given during three days. All are killed on the fifth day. A positive result for the urine is claimed if at least one animal shows ovarian changes, notably the presence of corpora lutea or corpora hemorrhagica. Positive results have been obtained with urine as early as in the fifth week of pregnancy.

The test will distinguish between true pregnancy and menopausal conditions, including "endocrine repercussions of abnormal emotional states." It is advisable to extract the urine with three times its volume of ether prior to injection in order to remove a toxic substance present in some urines, and fatal to mice.

The Friedman test only requires twenty-four to forty-eight hours, and for this reason is sometimes preferable. The relative merits of the three tests are discussed by Crew (1939).

ANDROGENS: MALE SEX HORMONES

The existence of chemical factors controlling secondary sexual characters in the male had long been inferred from the more obvious consequences of castration, before or after puberty. Early attempts by Brown-Séquard and others to obtain active extracts from testicular tissue were inconclusive, mainly for two reasons: the preparations were administered by the alimentary tract, from which the hormones are only absorbed with difficulty; and no exact method of chemical assay was available. Since 1930, Moore and others have shown that androgenic activity may be detected by the increased growth of the comb and wattles in castrated birds (capons); and Korenchevsky has based a method of assay on the increased growth of the prostate and seminal vesicles evoked when the hormone is injected into castrated rodents. Aided by these tests, four natural androgens have been isolated, and various artificial androgens have been synthesised, in addition to those from adrenal cortex.

Natural Androgens

Name.	Formula.	Source.	Discoverer.	Potency, in Rat Units.
Androsterone . .	$C_{19}H_{30}O_2$	Male urine.	Butenandt.	1 mg. = 1 r.u.
Dehydroandrosterone	$C_{19}H_{28}O_2$	Male urine.	Butenandt.	1 mg. = 0.3 r.u.
Testosterone . .	$C_{19}H_{28}O_2$	Testicle.	Laqueur.	1 mg. = 0.1 r.u.
Androstanedione .	$C_{19}H_{28}O_2$	Testicle.	—	—

Testosterone, $C_{19}H_{28}O_2$, the testicular hormone, is an unsaturated hydroxy-ketone derived from a parent steroid *androstan*, and appears in the urine as the two derivatives, *androsterone* and *dehydroisandrosterone*, both of which are much less potent.

A fourth androgen, *androstanedione*, $C_{19}H_{28}O_2$, also prepared by reduction of androsterone, has about one-third the potency of the parent compound.

Testosterone and androsterone can now be manufactured in quantity from cholesterol and other common sterols, which provide a more convenient alternative to the natural sources.

The androgens are all colourless crystalline compounds, sparingly soluble in water but readily soluble in fat solvents, and are usually administered by intramuscular injection of the solution in an oil.

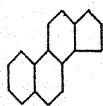
The international unit recommended by the League of Nations (1935) is the hormone activity of 0.1 mg. of crystalline androsterone,

as tested by a specific biological reaction. A 15 per cent. increase in the comb area may be expected from administration to a capon of one international unit daily for five days.

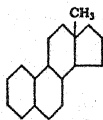
Effects of Androgen Injection.—In the castrated mammal there is a specific growth of accessory genital glands, the prostate, seminal vesicles and glands of Cowper, and a manifestation of the secondary sexual characters. The androgens differ somewhat in their results; testosterone, the most generally active, produces in capons twice as much comb growth as androstandiol and about six times as much as androsterone.

Significance of the Androgens.—Castration before puberty induces the eunuchoid state in which the secondary sexual characters fail to develop. Castration after puberty has little obvious effect other than subjective changes following the sterilisation. From this it might appear that the function of the androgens is limited to the maintenance of the reproductive efficiency of the individual. The isolation of androgens from the hormones of the adrenal gland, suggests that extra-testicular sources may be available in the adult.

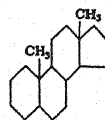
STRUCTURE OF THE SEX HORMONES



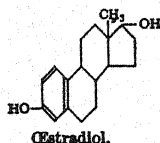
Cyclopentanophenanthrene
(parent steroid).



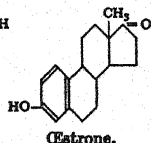
Estrane.



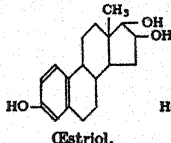
Androstane.



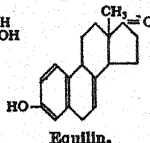
Estradiol.



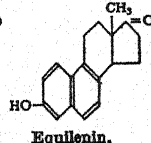
Estrone.



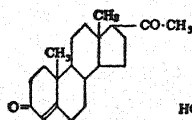
Estriol.



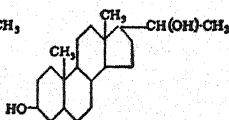
Equilin.



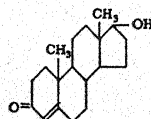
Equilenin.



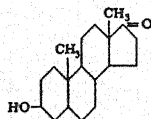
Progesterone.



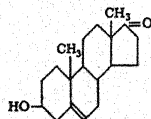
Pregnandiol.



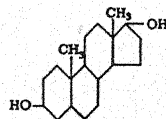
Testosterone.



Androsterone.



Dehydroandrosterone.



Androstandiol.

The Pituitary Control of Testicular Activity.—Gonadotropic hormones secreted by the anterior pituitary control (i.) the descent of the testicle, (ii.) the onset and maintenance of spermatogenesis, and (iii.) the output of the androgens, this last effect being homologous with that of FSH and LH in the female. Pituitary gonadotropic activity is slight before puberty, as shown by the absence of androsterone from the urine.

The oestrogenic female hormones (gynæcogens) are all derived from the 13-methyl steroid *œstrane*, $C_{17}H_{27}CH_3$. Progesterone, pregnandiol and the male hormones are all derived from the 10:13-dimethyl steroid androstane, $C_{17}H_{26}(CH_3)_2$.

PITUITARY HORMONES

The pituitary body, or hypophysis, is double in origin and multiple in functions. From the stomodæal pouch of Rathke is developed the anterior lobe and the *pars intermedia*; from the floor of the third ventricle are formed the *pars nervosa* of the posterior lobe, the *infundibulum* and *pars tuberalis*. The pituitary autacoids are of two types: secretions acting directly on other tissues, and *hormotropic* secretions acting indirectly by stimulating other endocrine glands.

A. Anterior Lobe

Six specific hormone proteins have been separated. The α (acidophil, or acid-staining) cells yield: (1) growth, (2) lactogenic, and (3) thyrotropic hormones; the β (basophil) cells yield the (4) follicle-stimulating, and (5) luteinising gonadotropins and (6) the corticotropic hormone. Anterior lobe extracts also display activities not yet referable to isolated factors; (7) ketogenic, (8) glycotropic, (9) glycostatic, (10) diabetogenic, and, in some animals, (11) pancreatropic, (12) parathyrotropic, (13) mammo-tropic effects.

(1) **Pituitary Growth Autacoid** (Van Dyke, 1930).—This factor stimulates general growth, particularly that of the osseous, connective, and epithelial tissues. It has no obvious action on the thyroid, the ovaries, or any other endocrine structure. It is lipoid in character, and is usually present in alkaline, but not in acid, extracts of the anterior lobe. Lack of the factor causes infantilism; excess causes gigantism and acromegaly, with overgrowth of hands, feet and jaws.

(2) **Prolactin**.—The growth of the mammary glands during pregnancy is ascribed to the action of the oestrogens; the actual onset of lactation is due to a pituitary factor, the effects of which can be demonstrated in the ovariectomised animal, showing that they

are independent of oestrogen once the mammary gland has developed. Prolactin accompanies LH in alkaline extracts of the anterior pituitary.

(3) **Thyrotropic Factor** (Crew and Wiesner, 1930) stimulates thyroid development in young animals, and regulates the secretion of the gland. Hyperthyroidism or hypothyroidism may result from excessive or inadequate output of the thyrotropic principle.

(4) **Oestrogenic Factor**, FSH, Prolan A (Zondek, 1930).—This evokes the secretion of oestradiol, and thus determines the onset of puberty, the maintenance of the menstrual cycle and the sequence of events in pregnancy. It is present in acid extracts of the anterior gland, and is excreted in the urine of pregnancy, along with the factor from the placenta (chorionic gonadotropin) that is responsible for the Aschheim-Zondek reaction. In males, the follicle-stimulant causes testicle growth and spermatogenesis.

(5) **Luteinising Factor**, LH, Prolan B (Zondek, 1930).—This evokes growth of the corpus luteum, secretion of progesterone, and inhibition of oestrogen output, thus inducing the second stage of the ovarian cycle.

In males, the hormone stimulates the interstitial cells of the testicle, and secretion of androgens.

(6) **Corticotropic Factor**. Hypophysectomy leads to atrophy of the adrenal cortex as well as atrophy of the thyroid. Pituitary hyperfunction, as seen in gigantism and basophil neoplastic growths in the gland, is often associated with cortical hypertrophy and with hyperthyroidism.

(7) *Ketogenic* and (8) *Glycotropic principles* are claimed to exist, and have been discussed along with carbohydrate metabolism (p. 324). The ketogenic principle is believed to stimulate the production of β -hydroxy butyric acid and the ketones found in diabetic urine. The glycotropic principle antagonises the effect of insulin, and consequently raises the level of the blood sugar. The *glycostatic factor* (9) enables hypophysectomised animals to maintain their muscle glycogen. The *diabetogenic factor* (10) represses carbohydrate tolerance and evokes glycosuria.

Significance of the Anterior Pituitary Gland.—By means of the gonadotropic hormones, FSH and LH, the gland regulates ovarian activity. By means of hormotropic factors, it co-ordinates the activity of the thyroid, adrenal and pancreas. By means of the growth factor and metabolic principles, it regulates development and general metabolism.

B. Posterior Lobe (*pars nervosa*)

(1) **Anti-diuretic Factor**, anti-diuretin.—Selective removal of the posterior pituitary evokes a polyuria due to inability of the renal

tubule to concentrate the glomerular filtrate. A very dilute urine is excreted, unaccompanied by any abnormal solutes, although the total sodium chloride output is increased in the early stages of the diuresis. Pathological pituitary dysfunction is associated with the disease diabetes insipidus, in which the urinary output may exceed 8-10 litres *per diem*. This leads to an acute dehydration of the organism and an attendant abnormal thirst, or *polydypsia*. Some experimental and pathological forms of the diuresis are rapidly abolished by injection of posterior pituitary extract. The recovery is transient, owing to destruction or excretion of the anti-diuretic factor. The factor has been separated from normal urine by Gilman and Goodman (1937), and shown to be distinct from the other pituitary principles. It is a true hormone, and is secreted by the gland in accordance with the need for water conservation.

(2) **Oxytocin**, α -hypophamine (Kamm *et al.*, 1928), causes a powerful contraction of the uterine muscle, and also stimulates the musculature of the bladder, the intestine, and the rest of the unstriated muscle of the organism, with the exception of that of the bronchi. A pseudo-galactagogue effect is also seen in the temporary outpouring of milk that follows injection of oxytocin or of pituitary extracts. This is ascribed to the expression of preformed milk from the gland owing to contraction of the ducts.

(3) **Vaso-pressin**, β -hypophamine, causes contraction of capillaries and arterioles after injection. The blood pressure slowly rises and remains high for several hours. There is little change in the rate and force of the heart beat other than that due to increased vascular resistance.

Injection of a second dose after the pressor effects of the first have worn off usually causes little or no response, the vascular system remaining insensitive for several hours. This may be due to the appearance of an anti-vaso-pressin in the organism, or, more likely, to the exhaustion of a contractile factor in the vessel walls. Birds for some unexplained reason appear to be immune from the vaso-pressor action of pituitary extracts. In man, the constricting effect on the capillaries is very conspicuous in the blanching of the skin that follows subcutaneous injection.

Apart from their therapeutic value, neither oxytocin nor vaso-pressin can be regarded as true hormones, since there is no evidence that they are employed in the regulation of physiological activity. Pregnancy and parturition are unaffected by removal of the posterior pituitary, and the normal uterine contractions during labour differ in character from those evoked by oxytocin. No marked circulatory disturbances follow removal of the posterior pituitary, and, although extracts containing vaso-pressin are active when

administered to anaesthetised animals, little or no pressor effect is observed in unanaesthetised dogs and human subjects. Commercial preparations of the posterior pituitary rich in one or more of the characteristic principles are represented by "puitritin," "pitocin" (oxytocin), "pitressin" (vaso-pressin), and "infundin."

These three hormones may be decomposition products of a parent poly-functional complex protein that is the actual hormone of the gland.

C. *Pars Intermedia*

(1) **Chromotropic Factor.**—The pigmentary system of amphibians is controlled by a hormone, *intermedin*, from the *pars intermedia*. Injection of extracts into adult frogs evokes a rapid darkening of the skin owing to dilatation of the pigment cells, or melanophores.

(2) **Metabolic Factor** (O'Donovan and Collip, 1938).—Even in thyroidectomised animals injection of *pars intermedia* extracts may stimulate metabolism, as shown by increased oxygen consumption and rise in body temperature. This is accompanied by a fall in the respiratory quotient, showing that there is a suppression of carbohydrate oxidation.

Hypopituitarism.—This may involve anterior or posterior lobe deficiency, or the entire gland.

(1) *Anterior Hypopituitarism*, as seen in maldevelopment or atrophy, is characterised by (i.) dwarfism, (ii.) sexual infantilism, (iii.) obesity, with lowered basal metabolic rate. The condition is profoundly modified by the fate of the thyroid gland.

(a) Fröhlich's syndrome, *dystrophia adiposo-genitalis*, occurs in children, and is due to infantile hypopituitarism and hypothyroidism. The victims are stunted, and often distorted by diffuse deposition of fat.

(b) Lorain's syndrome. Hypopituitarism unaccompanied by hypothyroidism. There is retardation of skeletal growth and absence of secondary sexual characters. Mental ability is unimpaired, and is often much above the normal. This type of infantilism is hereditary.

Hypopituitarism is frequent in old age, and marks a stage in senility.

(2) *Posterior Hypopituitarism.*—*Acute.*—There is no convincing evidence that the posterior lobe is essential for life. Complete removal is not followed by specific symptoms of deficiency. This paradoxical result may be due to (i.) readjustment of the organism during convalescence to a lower autacoid requirement, or (ii.) compensatory secretion by other endocrine organs, or (iii.) undetected persistence of sufficient *pars intermedia* tissue to provide for the requirements of life.

Chronic.—The only clinical condition ascribed to chronic

dysfunction is diabetes insipidus. Krogh has suggested that vasopressin is necessary for the maintenance of capillary tone, and that hypopituitarism is associated with low blood pressure, but Dale concludes that the adrenal medulla is the principal if not the exclusive factor in determining the condition.

Hyperpituitarism.—This may be of the anterior or posterior type, according to the tissue involved.

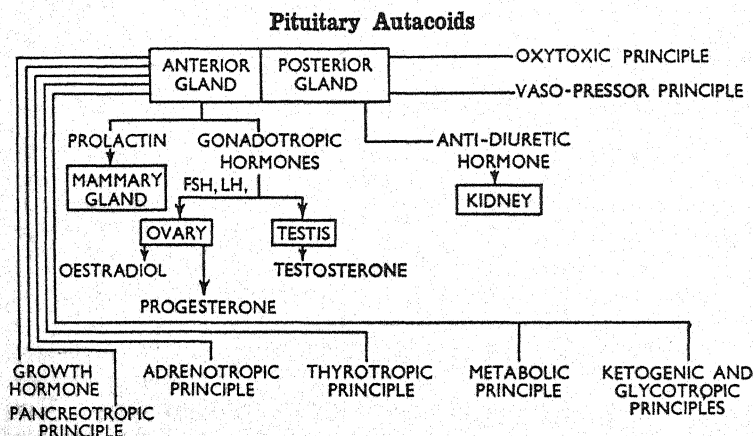
(1) *Anterior hyperpituitarism* :—

(a) Gigantism, due to early hyperpituitarism before the epiphyses of the long bones have united. The bones continue to grow uniformly, and the skeleton may reach a height of 7 or 8 ft.

(b) Acromegaly, due to late hyperpituitarism after the epiphyses have united. There is little change in the height of the skeleton, instead there is local overgrowth of the bones of the lower half of the face, enlargement of the hands and feet, curvature of the spine, and an approximation to the "gorilla appearance." There is hyperplasia of the connective tissue and thickening of the skin. The sexual potential is increased, but usually decays prematurely.

Both gigantism and acromegaly may be complicated by hypersecretion of the anterior lobe, leading to erratic glycosuria.

(2) *Posterior hyperpituitarism* has not yet been recognised as a clinical entity, although it may be involved in miscarriages and premature labour, and, perhaps, in some forms of anuria.

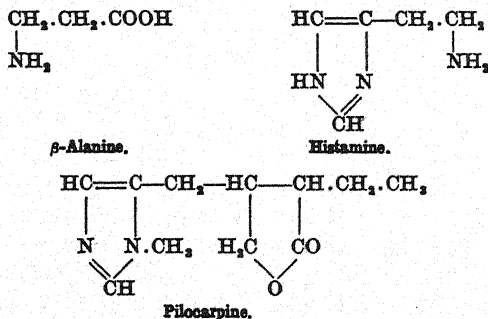


ALIMENTARY HORMONES

The events in the digestive process are controlled partly by the nervous system, and partly by hormones liberated by the action of the digestive products on the gastric and intestinal mucosa.

(1) **Gastrin**, gastric secretin.—In 1905, Edkins showed that acid extracts of the pyloric mucosa, when neutralised and injected into animals, evoked a secretion of gastric juice, an effect he attributed to the presence of a specific secretagogue, *gastrin*. Subsequently it was found that meat juice, meat extracts, protein digestion products and certain amino acids had a similar action, indicating that these factors might be of considerable importance in the maintenance of gastric digestion. The most potent secretagogue effect, however, was displayed by histamine and by β -alanine, neither of which are constituents of the native proteins. β -Alanine is a component of carnosine and of anserine, which occur in muscle tissue (p. 430), and appears to be responsible for the secretagogue property of meat extracts and broths. Histamine is readily formed from the natural amino acid histidine, by decarboxylation, and, according to Ivy (1933), is the actual gastric hormone present in pyloric mucosa, and is liberated during digestion.

Histamine is now employed for the purpose of obtaining human gastric juice for fractional analysis. About 0.1 mg. per 10 kg. of body weight is injected; gastric secretion begins within five minutes, and reaches a maximum between thirty to forty-five minutes after the injection. The juice evoked by histamine is rich in hydrochloric acid but deficient in enzymes, which indicates that the amine acts preferentially on the parietal cells. Injection of pilocarpine evokes a secretion rich in enzymes and mucin, and when administered along with histamine results in the production of a gastric juice similar to that secreted during normal digestion.



(2) **Secretin**, the secretory hormone for the pancreas, is found in the mucosa of the upper two-thirds of the small intestine. It can be extracted by acids, soaps, 70 per cent. alcohol, and 0.6 per cent. NaCl. All these methods have the disadvantage that they yield a product highly contaminated with vaso-dilators, during the subsequent removal of which most of the secretin is lost. The purified

autacoid is a white powder containing 6·7–8 per cent. N, and is a proteose of m.wt. 5,000. When injected intravenously it causes a rapid secretion of pancreatic juice, and also has a secretagogue effect on the liver and the glands of the small intestine. The action is not species specific, and somewhat resembles that of pilocarpine, but the pancreatic secretion evoked by secretin is richer in alkali and poorer in enzymes than the secretion obtained by vagal stimulation or the use of a vagomimetic drug, such as pilocarpine.

Secretin Distribution in Alimentary Mucosa of the Cat, expressed in Volume of Pancreatic Secretion evoked by a Standard Dose

Region.	Secretion.
	ml.
Stomach, fundus . .	0·0
„ pylorus . .	0·0
Small intestine :	
Upper third . .	2·75
Middle third . .	2·6
Lower third . .	0·5
Ascending colon . .	0·2

The hormone is inactive when given by the mouth, which greatly restricts its therapeutic application.

Secretin is liberated by the presence of bile salts, fats, or to a much lesser extent, free acid, in the upper small intestine.

The secretin unit suggested by Ivy is "that amount of dried material in solution which when injected will cause a 10-drop (0·4 ml.) increase in the rate of flow of pancreatic juice within a ten-minute period following the time of injection, as compared with the previous preceding ten-minute period."

The animal used for the purpose of assay is the cat.

(3) **Cholecystokinin**, a hormone evoking contraction of the gall bladder, resembles secretin in its distribution, and is present in extracts of the intestinal mucosa. Separation of the two hormones has been claimed by Ivy and Oldberg (1928).

(4) **Enterogastrone**.—A factor inhibiting gastric motility and secretion is, according to Ivy (1937) set free by the presence of fat in the small intestine.

(5) **Villikin**, released by action of acid on duodenal mucosa, and also present in yeast, stimulates contractile pulsations in the intestinal villi.

Anti-hormones.—After repeated injection for some time, a hormone may fail to evoke its typical response. This has been attributed by Collip to the production of a corresponding anti-hormone by the organism. The effect often may be due to an antagonism developed towards protein contaminants accompanying the hormone extract (Thompson, 1941).

MISCELLANEOUS AUTACOIDS

Extracts of animal or plant tissues when injected into higher animals often display a vaso-motor effect owing to the presence of *vaso-dilators* acting on arterioles and capillaries. Some of these vaso-dilators appear to be artefacts formed during the preparation of the tissue extract, but at least six are believed to exist as biological reactants, and may be regarded as typical hormones. These are: (1) Histamine, (2) acetyl choline, (3) choline, (4) adenylic acid, (5) adenosine, and (6) hypertensin.

(1) **Histamine** has been identified in extracts of intestinal mucosa, liver, lung and posterior pituitary. It displays three characteristic effects :—

- (i.) A secretagogue action on the lachrymal, salivary, gastric and pancreatic glands.
- (ii.) Contraction of involuntary muscle, notably uterus, intestine and bronchioles.
- (iii.) Fall in blood pressure owing to generalised capillary dilatation and paralysis, accompanied by increased capillary permeability and transudation of plasma. In the human subject, a subcutaneous injection of 0.3 mg. of histamine evokes a marked fall in diastolic pressure, a rise in pulse rate and temperature, and a flushing of the skin. Arteriolar dilatation accompanies the capillary response in man, dogs and monkeys (p. 416).

(2) **Acetyl choline**, the most powerful reactant known to occur in the animal body, has been identified in fresh spleen by Dale and Dudley, and also occurs as the neurocrine liberated locally when parasympathetic (cholinergic) nerve endings transmit impulses. Characteristic effects following intravenous injection are :—

- (i.) Fall in blood pressure owing to a direct dilator action on peripheral blood vessels. This effect is observed even in doses of 1×10^{-5} mg., and is antagonised by atropine.

The direct vascular effect is accompanied by cardiac inhibition equivalent to vagal stimulation (p. 420).

- (ii.) **General Cholinergic Phenomena.**—These are similar to the effects of parasympathetic stimulation, and include

lachrymal, salivary, gastric and pancreatic secretion, increased motility of the alimentary tract, and contraction of the bladder.

Acetyl choline is transient in effect owing to its rapid hydrolysis by the widely distributed enzyme, choline esterase, which resolves it into free choline, with a potency about 100,000 times less than the acetyl ester.

(3) **Adenylic acid**, present in extracts of skeletal and cardiac muscle, and in brain, kidney and spleen, is derived from adenosine triphosphate, the phosphate carrier in glycogenolysis and glycolysis. Adenylic acid, and its nucleoside component adenosine, are active vaso-depressors, evoking general arterial dilatation accompanied by cardiac retardation. The coronary arteries are dilated, and for this reason preparations of the autacoid ("lacarnol") have been used in the treatment of angina pectoris. Accumulation of adenylic acid in the tissues is prevented by its conversion into the less potent nucleotide inosinic acid.

Adenylic derivatives appear to constitute the "muscle shock factor" released from crushed or ischæmic muscle (Green *et al.*, 1943).

(4) **Renin**.—Goldblatt (1942) has shown that persistent vascular hypertension develops in dogs when the blood supply to the kidney is restricted. A factor, *renin*, has been separated from extracts of ischæmic kidney cortex. It is an enzyme, and acts on a globulin present in normal blood plasma, yielding the pressor agent, *angiotonin*, or *hypertensin*, responsible for the renal effect.

Hypertensin is non-colloidal and thermostable. It is destroyed by an enzyme, *hypertensinase*, present normally in blood plasma. Renin, unlike the other pressors, adrenaline, "puitritin," and tyramine, does not decrease peripheral blood flow, and lower skin temperature, on intravenous injection.

Testicular Diffusing Factor.—Aqueous extracts of mammalian testicle contain a substance that greatly increases skin permeability to injected fluids, and the injection spreads rapidly through the dermis. Intracutaneous injection of saline, serum or extracts of any tissue other than testicle cause a bleb, the margins of which persist for twenty to thirty minutes.

The factor is associated or identical with the enzyme, *hyaluronidase*, a *mucinas*e found in bacteria, leeches, snake venoms and bee sting extracts. It liberates acetylhexosamine and an aldobionic acid from *hyaluronic acid*, the protective carbohydrate in skin.

Lysozyme is a crystallisable heat-coagulable protein present in raw egg-white, saliva, nasal and lachrymal secretions, and may be identical with the anti-biotic *avidin* (p. 289). Lysozyme attacks

and dissolves certain bacteria by hydrolysing their cell substance and releasing acetyl hexosamine.

PLANT AUTACOIDS, PHYTOHORMONES

Name.	Source.	Function.
(1) Bios complex . . .	Yeast extracts, bran, etc.	Growth factor.
(2) Auxin <i>a</i> . . .	Apical tissue of shoots, etc. Pollen.	Cell elongation. Phototropism. Geotropism.
(3) Auxin <i>b</i> . . .	Plant extracts.	Growth factor.
(4) Indole-3-acetic acid	Urine, etc.	Root formation.
(5) Traumatic acid .	Injured tissue.	Wound repair.

(1) **Bios Complex.**—In 1901, Ide and his pupil Wildiers showed that a water-soluble factor, termed *bios*, is necessary for the growth and development of many, but not all, strains of the yeast *Saccharomyces cerevisiæ*. Bios occurs plentifully in yeast extracts, plant leaves, bran and the outer coats of seeds. Various compounds have been isolated from the mixture of substances present in bios extracts, and their specific potency has been accepted or denied by different workers.

These conflicts of opinion are now regarded as due largely to the differences in species effect, which have led to ambiguous or contradictory results. The growth of all monocellular organisms depends on the elaboration of cyto-skeletal compounds, and growth rate can be increased when some at least of these are provided by the environment.

A bios may be defined as a growth factor for monocellular organisms that can be derived both from endogenous and exogenous sources. Representative bios substances are : (i.) *D*-inositol, or bios I. ; (ii.) nicotinic acid, which is necessary for the growth of *Staphylococcus* and other bacteria ; (iii.) uracil ; (iv.) vitamin B₁ ; (v.) pantothenic acid, a polyhydroxylic acid derived from β -alanine, and necessary for yeast growth, (vi.) biotin, and (vii.) folic acid, found in green leaves, yeast, and many animal tissues (Mitchell *et al.*, 1941).

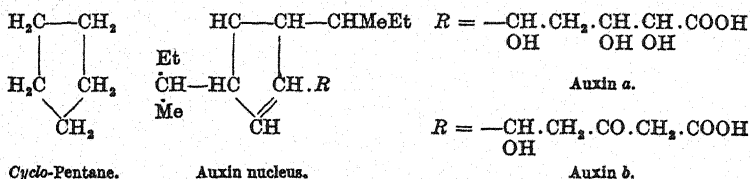
(2) **Auxins.**—Plant development is attended by elongation of individual cells, as distinct from their multiplication, and by resulting movements of stems towards the light (phototropism) or roots towards the soil (geotropism). All these changes have been shown to be due to hormones, termed auximones or auxins, secreted in the apical regions and spreading by diffusion.

The study of auxins arose, in 1910, with the work of Boysen-Jensen on the coleoptile, or primary leaf sheath, of grasses, and was extended

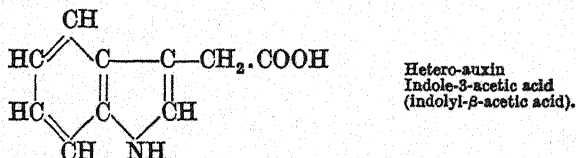
by Went, and other investigators; the isolation and identification of the auxins being due to Kögl and his colleagues (1931-37).

Auxin *a* and auxin *b*, the principal phytohormones, are obtained from the ether-soluble fraction of lipid extracts of rapidly growing apical tissues of roots and tips, and are assayed in terms of their ability to evoke renewed growth in decapitated coleoptiles (auxin *a*) or in moulds (auxin *b*). Auxin *b* is readily destroyed by both acids and alkalies, and thus can be separated from auxin *a*, which is relatively acid-stable, but decomposed by alkalies.

Both the auxins are derivatives of a *cyclo*-pentane ring similar to that forming part of the steroid nucleus.



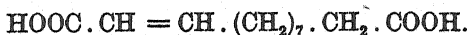
(3) **Hetero-auxin, Indole-3-acetic Acid.**—In the survey of plant and animal extracts for substances having an auxin effect, it was found that urine was very rich in a growth-promoting factor, which on isolation proved to be chemically unrelated to auxin *a* or *b*, and was termed hetero-auxin. It was subsequently identified as an indole derivative of acetic acid, and is derived from tryptophane by bacterial decomposition in the intestine.



The effect of the phytohormones is non-specific as regards species; auxin *a* stimulates growth in a great variety of plants; auxin *b* has no action on coleoptiles, but promotes growth and mycelium formation in *Aspergillus niger*, and other moulds; hetero-auxin appears to be a general stimulant for root formation. None of these hormones has been found to influence cultures of animal or malignant tissues, although there is a quantity of miscellaneous evidence that animal hormones, notably the oestranes and thyroxin, can promote plant growth.

(4) **Traumatic Acid**, the plant wound hormone, is liberated by damaged tissues, and stimulates growth of parenchymatous cells

in the repair process. It has been isolated by English (1939) and shown to be 1 — decene — 1, 10 — dicarboxylic acid,



ORGANISERS AND EVOCATORS

Embryonic development involves two processes: *differentiation* and *organisation*. As growth proceeds, various territories of the segmented egg lose their common primordial character, and acquire the morphological and chemical features of tissues and organs. "Viewed from the community of parts, this is differentiation; viewed from the individual parts, it is specialisation" (Weiss, 1935). These processes of differentiation or specialisation are accompanied by organisation, whereby the emergent structures are arranged in accordance with the species pattern of the individual. Formerly, embryonic development was popularly and vaguely attributed to a teleological guidance or purpose latent in the embryo, but since 1921 the work of Spemann, Mangold, Waddington and the Needhams has shown that it depends on the activity of chemical factors, or *organisers*, elaborated locally and exerting an *inductive* effect by diffusion into neighbouring sensitive areas, or *fields*. Induction may occur naturally during the growth process or be brought about artificially by means of grafts. Two types of graft induction can occur: *evocation*, in which the graft carries no field of its own, and merely activates a field in the affected region of implantation; and *induced organisation*, in which the graft carries its own field and imposes it on the host. Under appropriate conditions, induction may be brought about by tissue extracts, as well as by fragments of living tissues, the effect being ascribed to the presence of evocators and organisers, which may be defined provisionally as morphogenetic autacoids determining embryonic development.

In the earliest stages of the developing vertebrate embryo the constituent cells have little individuality, and can give rise almost to any embryonic structure. As soon as the stage of gastrulation is reached, however, the cells become individualised, and can only give rise to particular tissues. This is due to an influence exerted on the rest of the embryo by a specialised group of cells in the region of the dorsal lip of the blastopore, which produce an organising autacoid. Removal of these organiser cells leads to cessation of normal differentiation; micro-injection of the extract of the disintegrated cells induces differentiation. It is likely that different organisers are manufactured for different purposes in embryonic development. The amphibian factor, studied by Waddington and

the Needhams, appears to be a steroid. It induces the formation of the neural tube and other neural tissue in the early embryo of the newt.

Polyploidogens.—The dried ground-stem, or corm, of the autumn crocus, or meadow saffron (*Colchicum autumnale*), used empirically in treatment of gout, contains about 0.2 per cent. of a phenanthrene derivative, *colchicine*, $C_{44}H_{25}O_6N$. Colchicine acts as a nuclear poison, or caryotoxin, in plants and animals, and retards cell-division in the later stages of mitosis. The split chromosomes fail to separate, and the resulting daughter-cell shows polyploidy, or abnormal chromosome equipment. By this means it is possible to evoke chemical mutations, and produce new varieties of plants. Acenaphthene, β -naphthol, and some related cyclic hydrocarbons have a similar effect.

Anti-biotics are specific compounds secreted by lower organisms, and capable of inhibiting the growth of other species (bacteriostatic effect), or even poisoning (bacteriocidal effect) or dissolving (lysis effect) foreign organisms. They are important weapons in the biological warfare that characterises life at the lower levels in the biosphere, and some of them are of great value in animal therapy. Thus, in 1928, Fleming observed that the growth of a staphylococcus colony on a solid medium was checked by lysis in the zone surrounding an accidental infection by a common mould, identified as *Penicillium notatum*. The anti-biotic, later named **penicillin**, was extracted, and found to be antagonistic to staphylococci, streptococci, pneumococci, and some other bacteria, though not to *B. coli*. Penicillin is active in a dilution of 1 in 50×10^6 , and is one of the most powerful anti-biotics yet discovered.

From the sporogenic soil organism, *B. brevis*, Dubos (1941) has isolated two crystalline peptides, *gramicidin* and *tyrocidin*, both of which are powerfully bactericidal for gram-positive and other organisms, but too toxic to higher animals to be of therapeutic use. Half the amino acid units in gramicidin occur in the uncommon form of D-isomers. Anti-biotics appear to act by becoming entangled in the metabolic mechanism of the foreign organism, and thus depriving it of some essential enzyme or nutrient.

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CHAPTER 25

THE INTERNAL ENVIRONMENT: BLOOD AND TISSUE FLUIDS

THE immediate purpose of life is survival. After a period of parasitic growth, the animal reaches a stable form capable of maintaining a biological relationship with its environment. The increasing specialisation of function associated with ascent of the evolutionary scale demands an increasing complexity of organisation and co-ordination which reaches its highest level in the human species, the most elaborate apparatus yet known in cosmic history.

But however multifarious and cryptic the activities of life, one general principle governs all physiological processes; organic existence demands stability in the composition of the internal environment, which in the higher animal is represented by the blood, and the cerebro-spinal and other tissue fluids. This fundamental law has been expressed by Claude Bernard, in a well-known epigram:—

La fixité du milieu intérieur est la condition de la vie libre.

As Barcroft has shown, the freedom of human life, as expressed in repose and action, requires constancy in the composition of the blood and the cerebro-spinal fluid, while the stability of the internal environment as a whole requires inter-dependent mechanisms for storage, distribution and removal of solutes. Transitory changes

Effects of Changes in the Human Internal Environment

Deficiency. ←	Internal Environment.	→ Excess.
Hypopyrexia. Collapse. Anoxæmia. Unconsciousness. Alkalosis.	Temperature.	Hyperpyrexia. Delirium.
	Oxygen.	
	H-ion concentration.	Acidosis. Coma.
Dehydration. Thirst. Asthenia.	Water.	Edema.
	Sodium chloride.	Thirst. Paresis.
Hypoglycæmia. Collapse.	Glucose.	Hyperglycæmia. Glycosuria.
Hypocalcæmia. Convulsions.	Calcium	Hypercalcæmia. Atonia, coma.

in the blood due to metabolic activity are compensated for by specially adapted systems for neutralisation, detoxication, and pulmonary and renal excretion, and form part of the routine physiological activities of life. Excessive changes in the blood composition are the result of pathological processes, resulting in abnormal metabolism or in defective compensation and excretory dysfunction.

WATER BALANCE

The animal body can be regarded as having three water-permeable compartments, containing, respectively, blood plasma, interstitial fluid, and intracellular fluid. An osmotic pressure of approximately 6.9 atm. is maintained throughout, and is regulated by the kidneys, which alter the composition of the urine as required. The volume of the circulating blood is kept within normal limits by the non-diffusible proteins of the plasma, which maintain an independent osmotic pressure of 0.03 atm. within the vascular system, in addition to the osmotic pressure due to the diffusible, non-colloidal solutes of the blood.

Representative Water Distribution

65 kgm. Man

Tissue.	Wt. in km.	Per cent. body weight.	Water content.	
			Per cent.	kgm.
Muscle . . .	27.1	41.7	75.6	20.5
Skin . . .	11.7	18	72	8.4
Blood . . .	5	8.5	80.5	4.1
Skeleton . . .	10.3	15.9	22	2.27
Liver . . .	1.5	2.3	68.2	1.0
Brain . . .	1.4	2	74.8	0.97
Lungs . . .	0.4	0.7	78.9	0.35
Heart . . .	0.3	0.5	79.2	0.24
Kidneys . . .	0.24	0.37	82.7	0.2

Total water content, or total fluid volume, is found in the living animal by injecting a known amount of some freely diffusible substance, such as water containing D_2O and DHO , and, after it has had sufficient time to be distributed through the body, estimating its dilution in a known volume of plasma.

Extracellular fluid volume is found by injecting an inert solute, such as sucrose or SO_4^{--} , that is capable of escaping from the blood stream, or vascular compartment, but is incapable of entering the tissue cells. *Intracellular volume* is calculated by subtracting the value for extra-

cellular volume from that of the total fluid volume. *Plasma volume* is found by dye-injection methods.

All these procedures are liable to errors due to escape of the indicator through the kidneys during the period of 15 to 30 minutes required before uniform distribution has been attained. The results lead to the general conclusion that plasma volume is about one-third of the interstitial fluid volume, and about one-tenth of the intracellular fluid volume.

Water Depletion.—Water is continuously leaving the body in the expired air of the lungs and the “insensible” perspiration of the skin. The output is almost constant and, in adults, is 300 to 500 ml. *per diem*, depending on the surface area of the body, and the temperature and moisture content of the environment.

In addition, there is the daily output of 800 to 1,500 ml., by the kidneys, and 50 to 200 ml. by the intestine; together with the extra water lost by “sensible,” or visible, perspiration in response to bodily activity.

Whenever water intake is inadequate, or water output is excessive, the water balance of the organism is displaced towards the side of dehydration, the first symptom of which is thirst.

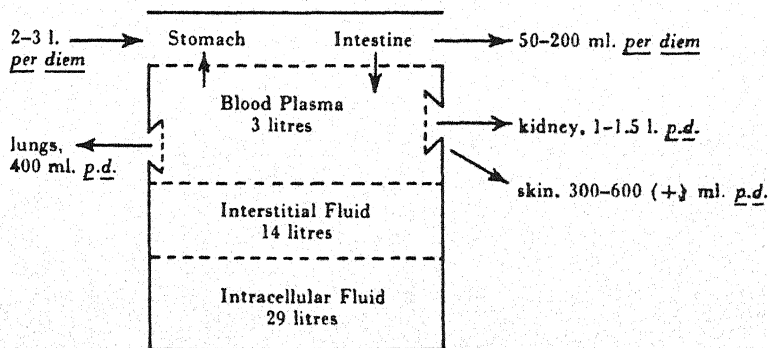
That thirst is due to a rise in the osmotic pressure of the blood, irrespective of the total amount of water in the body, is shown by the effect of injecting 500 ml. of *N.NaCl*, which in a few minutes reduces the saliva output to one-tenth, or less, of the usual value of 1 ml. per minute, thus causing dryness of the mouth. The thirst sensation may arise from a sensitive zone in the pharynx (Cannon, 1918), or may be one of the responses of the organism to general dehydration (Gilman, 1937). That it normally is due to a local stimulus is shown by its temporary abolition, when the back of the mouth is anaesthetised, or when salivary flow is evoked by sialogogues, such as pilocarpine, that do not lower the osmotic pressure of the plasma. Pathological thirst, such as follows excessive loss of water by the kidneys, in diabetes insipidus, or by the intestine, in cholera, is not relieved by sialogogues. It accompanies and reveals the serious dehydration of the entire body, in which the muscles and cutaneous tissues may lose 10 to 20 per cent. of their total water. Collapse finally ensues from fall in blood volume due to transfer of water from plasma to tissues. Occupational dehydration is experienced by deep-level miners, stokers, and others engaged in hard work in warm surroundings. Large quantities of sweat are secreted to cool the body, and thus both water and sodium chloride are lost. If this type of dehydration be countered by the obvious method of drinking plain water, the osmotic pressure of the body fluid is lowered abruptly, and water-intoxication results, with nausea and muscular cramp. The proper treatment is the use of normal saline as a beverage to replenish body salt as well as body water.

Vascular Shock.—Any rapid reduction in total blood volume predisposes to the syndrome of vascular shock, which includes fall in blood pressure and body temperature, weak and rapid pulse, pallor, apathy and collapse. When the fall in blood pressure is due

to hæmorrhage, compensatory transfer of fluid from the interstitial reserves takes place, and may avert death. Vascular shock, however, can come from abnormal loss of fluid by the blood, owing to increase of capillary permeability by release of histamine, adenylic acid, or similar depressor compounds, from injured tissues. The rapid restoration of blood volume is essential in the treatment of any form of vascular shock, and Scudder (1940) emphasises the importance of using the correct saline transfusion fluids in the process.

Water Retention.—Rise in osmotic pressure of the plasma and tissue fluids is compensated by excretion of more solutes in the urine; fall in osmotic pressure, conversely, results in the excretion of a urine sufficiently dilute to remove the surplus water from the organism. If, however, the load of water or of salt ingested exceeds the clearance capacity of the kidneys, fluid accumulates in the body. Partial failure of the kidney to excrete salt or water, even when the diet is normal, is shown by the progressive retention of surplus body fluid.

In order to avoid plasmolysis and disintegration of tissue cells, the osmotic pressure of the body fluid must be kept at its normal level, hence any retention of water must be accompanied by a corresponding retention of Na^+ and Cl^- , and any retention of NaCl demands retention of sufficient water to keep it at isotonic concentration, equivalent to 6.9 atm., or 0.9 per cent. NaCl . The renal output of water is controlled by the anti-diuretic factor of the anterior pituitary gland; the Na^+ and Cl^- output is controlled by the hormones of the adrenal cortex. Apart from adrenal dysfunction, with accompanying excessive excretion of NaCl , water depletion is generally due to the inability of the organism to retain water; while water retention is generally due to inability to excrete sufficient salt.



Water Distribution in 70 kgm. Man.
(Modified from Gamble, 1937.)

Sweat.—The *eccrine* sweat glands distributed generally over the body surface, and the *apocrine* glands of the axilla and groin secrete a very dilute fluid of varying composition and reaction, the primary function of which is to facilitate heat-loss by evaporation. The glands are innervated by cholinergic fibres from the sympathetic supply, and the output is evoked by rise in temperature, exercise, the emotional release of hormones, and by drugs such as pilocarpine, and may reach a maximum of 1-2 l. per hour, as visible, or "sensible," perspiration.

The average composition of human sweat, in mg. per 100 ml., is: total solids, 250-700; inorganic solutes, 200-500; NaCl, 100-370 (Kuno), 500 (Dill); lactic acid, 70-160; urea, 20-46; K, 14-39; Ca, 22. Traces of reducing substances and volatile aromatic compounds also occur, especially in apocrine sweat. Sweat as freshly secreted is alkaline (pH 7-7.4), but in contact with the skin becomes acid (pH 5.4).

The physiology of human perspiration is reviewed by McSwiney (1935), Kuno (1935) and Dill (1938).

BLOOD

The vascular apparatus in mammals, according to Drinker (1942), has five essential features:—

(1) A closed system of blood capillaries with endothelial walls of varied permeability, but capable of retaining almost all of the blood plasma during the usual conditions of rapid transit.

(2) A variable hydrostatic pressure in the capillaries.

(3) The presence of extra-cellular non-respiratory proteins in the plasma.

(4) An extra-vascular tissue fluid lower in protein content than the blood plasma, but otherwise almost identical.

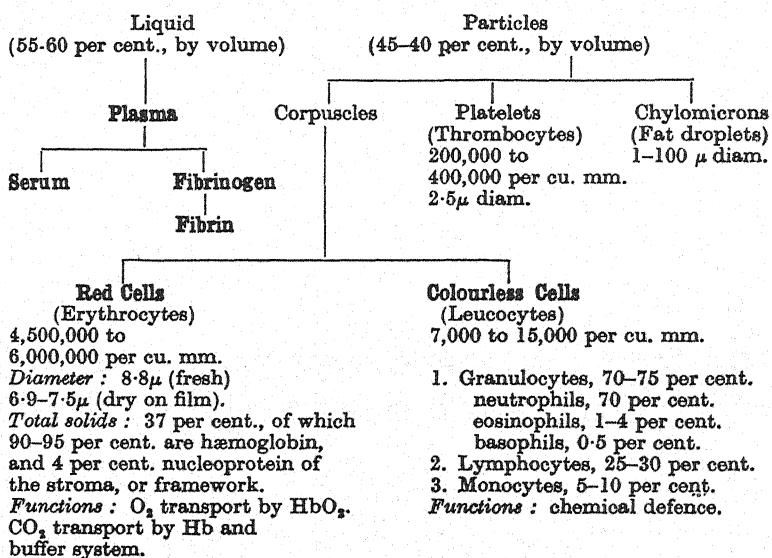
(5) A closed system of lymphatic capillaries with very permeable endothelial walls, which lack an inherent mechanism for moving their contents into the larger, valved lymph vessels, and depend on inconstant external changes, such as muscular contraction, for the uptake of fluid and the eventual flow of the lymph stream back to the general circulating blood.

Blood is a fluid tissue consisting of corpuscles and other particles dispersed in a colloidal medium, the *plasma*. It is opaque, owing to the enormous number of cells in suspension, and red in colour, owing to the pigmented corpuscles, or erythrocytes, which carry the hæmoglobin. Human plasma has no hæmoglobin, and is a clear liquid with a faint yellowish tint, due to traces of carotene and xanthophyll, derived from the diet, and bilirubin, from degraded hæmoglobin.

On dilution with water or hypotonic salines, the erythrocytes swell and disintegrate, liberating hæmoglobin, and thus forming a red,

transparent solution, "laked blood." *Hæmolysis*, or dissolution of the red corpuscles, can also be effected by soaps, saponins, fat-solvents, and some snake venoms and bacterial toxins. When hæmolysis occurs in the living animal, the liberated hæmoglobin may be excreted by the kidney, giving rise to hæmoglobinuria.

When blood is centrifuged, or allowed to remain under conditions unfavourable for clotting, the red cells (sp. gr. 1.09) separate from the lighter plasma (sp. gr. 1.03), and are found to have made up 40 to 45 per cent. by volume of the original blood. For accurate measurement of this important ratio, a narrow graduated centrifuge tube, or *hæmatocrit*, is used.



Composition of Human Blood.

Physical Characteristics of Human Blood.—*Specific gravity*, 1.040-1.066 (water = 1.000). *Reaction*, slightly alkaline (arterial blood or plasma, pH 7.3-7.4 ; venous blood or plasma, pH 7.3-7.5 ; cell contents, pH 7.1). *Osmotic pressure*, entire blood or plasma, 6.9-7.0 atmospheres ; plasma colloids, 0.03 atms. (23-28 mm. Hg.). *Viscosity*, entire blood, 3.6-5.3 ; plasma, 1.7-2 (water = 1.0). Specific gravity of blood or plasma can be found by observing if a drop sinks or floats in an inert liquid of known sp. g., such as can be made by mixing chloroform (sp. g. 1.527) and benzene (sp. g. 0.874). When a mixture has been obtained in which the drop of blood remains poised, its specific gravity is measured by a hydrometer, and is equal to that of the blood. A more convenient method for clinical work was devised by Barbour and Hamilton (1926), in which the sp. g. is found from the time of fall of a drop of blood down a column of liquid of known density.

The specific gravity of plasma depends chiefly on its colloid content, and gives an accurate index of protein concentration.

The reaction of blood is found by means of a hydrogen or glass electrode, precaution being taken to avoid escape of CO_2 from the blood during manipulation.

Total Volume.—Knowing either the total volume of the plasma or the cells, it is possible to calculate the total volume of the blood in the body from the plasma/cell ratio found by the hæmatocrit. Results by different methods are at variance, but sufficiently consistent to lead to the conclusion that the total blood volume amounts to 7–8 per cent. of the entire body weight, or 70–80 ml. per kgm. A 70 kgm. adult has about 5 litres of blood in his body.

Plasma volume is found by injecting a known amount of a harmless dye, such as "vital red" or "Evans blue," which can neither enter the corpuscles nor escape by the capillaries. After 15–30 minutes, to allow for uniform distribution, a known volume of blood is withdrawn, and the dilution of the dye in the plasma is estimated colorimetrically. Error may arise from excretion of some of the dye by the kidneys during the experiment.

Total red cell volume may be found by two independent methods: (a) *Carbon Monoxide Fixation*, introduced by Haldane (1900), and modified by subsequent workers, including Chang and Harrop (*J. Clin. Invest.*, 1928, 5, 329). Air containing a known but harmless amount of CO is rebreathed until all the CO has been absorbed by the hæmoglobin. After allowing time for dilution, a sample of blood is withdrawn, and its degree of saturation with CO is found by a reversion spectrometer. From this, the total quantity of hæmoglobin required to account for all the CO absorbed is calculated. Then, knowing the percentage of hæmoglobin in the corpuscles, the total red cell volume is obtained.

(b) *Isotope Dilution*. When radioactive iron, ^{59}Fe , is given to anæmic dogs, it is used in formation of new hæmoglobin, and the cells thus labelled do not discharge or exchange the isotope during their lifetime. When such blood, containing a known amount of isotopic hæmoglobin, is injected into another animal of the same species and blood type, uniform distribution of the labelled red cells takes place within ten minutes of the injection, and remains unchanged after three days.

By measuring the concentration of the isotope in a sample of the blood the total red cell volume can be calculated (Hahn *et al.*, *Science*, 1941, 93, 88). Radioactive phosphate can be used in a similar manner for the measurement of total body fluid.

Sedimentation Rate.—From the size and density of the red cells, a sedimentation rate of about 0.2 mm. per hour is required by Stokes's Law (p. 76). Owing to agglutinating factors associated with the plasma proteins, the rate of fall is much greater. Values obtained by Fåhræus (1929), in mm. per hour, are: new-born child, 0.5; normal man, 3.3; woman (non-pregnant), 7.4; woman (pregnant), 44.9.

RESPIRATORY FUNCTIONS OF THE BLOOD

(1) **Oxygen Transport.**—The oxygen reserves of man are about 800 ml., as HbO_2 , in the blood; 400 ml., in the supplemental and residual air in the lungs; not more than 70 ml., dissolved in tissue and body fluids; and, perhaps, 50 ml., in muscle hæmoglobin. Altogether, this represents only about 3 to 4 minutes' supply for the body at rest, and can have no survival value for life (Barcroft, 1932).

The air in the lung alveoli is, approximately, at atmospheric pressure (760 mm. Hg), and contains about 14 per cent., or one-seventh of its volume of oxygen, which, therefore, has a partial pressure of $1/7$ atms., or 108 mm. Hg. This diffusion pressure enables O_2 to enter the pulmonary capillaries and pass into the red cells.

Composition in ml. per 100 ml. of gas or liquid

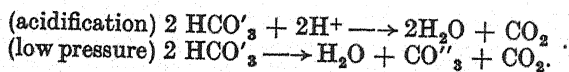
Gas.	Atmospheric Air.	Alveolar Air.	Arterial Blood.	Venous Blood.	Alveolar Air.	Expired Air.
O_2	20.93	14-15 →	19	13	14-15	16.9
CO_2	0.03	5.5	45-50	50-60 →	5.5	3.5
N_2	79.4	80	1	1	80	79.5

At 36°C ., and a partial-pressure of $1/6$ to $1/7$ atm., the quantity of O_2 that can dissolve in blood plasma is not more than 0.36 ml. per 100 ml. Arterial blood, however, contains nearly 20 ml. O_2 per 100 ml., which can be released and measured by a vacuum pump, or displaced by ferricyanide. When blood is exposed to an O_2 pressure of 145 mm. Hg, it becomes fully saturated with oxygen, all the available corpuscular hæmoglobin being in the form of HbO_2 . Any further increase in O_2 pressure merely increases the small amount of the gas that is dissolved in the plasma. Consequently, blood leaving the lungs by the pulmonary veins is nearly 100 per cent. saturated, as regards its oxygen capacity. As it circulates through the body, it comes into equilibrium with the tissue fluids, in which the O_2 pressure is very low, because of the demands by tissue respiration systems. With the fall in O_2 pressure, the HbO_2 dissociates, until the blood is about 60 per cent. saturated, which is the ordinary level of venous blood, and represents an O_2 content of 12 ml. per 100 ml. Dissociation of the HbO_2 is also promoted by the decreasing alkalinity of the blood, which changes from pH 7.45 to pH 7.35, owing to entrance of CO_2 from the tissues.

(2) **Carbon Dioxide Transport.**—In plasma and other aqueous liquids, carbon dioxide may be present as: (a) dissolved CO_2 , (b) hydrated CO_2 , or carbonic acid, H_2CO_3 , (c) acid carbonate ions, HCO'_3 , and (d) carbonate ions, CO''_3 . All four exist in an equili-

brum, the composition of which depends on the pH and the gaseous pressure of CO_2 to which the solution is exposed. Carbonic acid is moderately strong ($K_1 = 2 \times 10^{-4}$), and ionises almost instantaneously, but the hydration of CO_2 to H_2CO_3 , or the converse dehydration of H_2CO_3 , is slow, unless catalysed by the enzyme *carbonic anhydrase*, which accompanies Hb in the red cells, but does not occur in the plasma.

When plasma is acidified it yields 40–60 ml. CO_2 per 100 ml., but when it is exposed to zero CO_2 pressure in a vacuum pump, only about half its total CO_2 is released. In these respects, plasma resembles an aqueous solution of HCO_3^- :



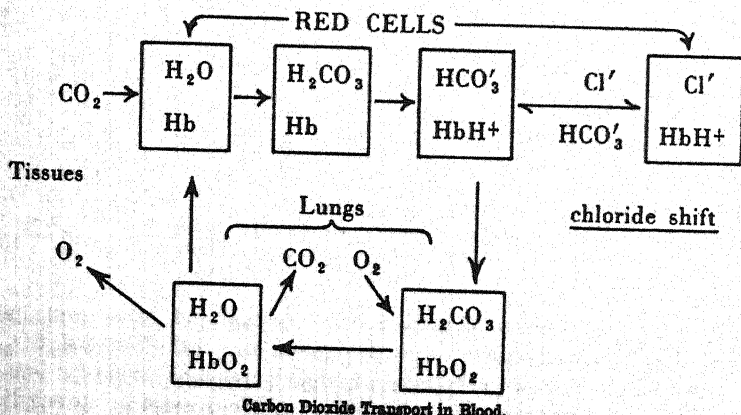
At the pH of blood, 7.4, and at a partial pressure of 40 mm. Hg, which corresponds to the CO_2 content of alveolar air, only 2.7 ml. of CO_2 is present in solution in the plasma, the rest is nearly all in the form of HCO_3^- .

Entire blood resembles plasma in yielding all its free and ionised CO_2 on acidification; but, unlike plasma, it also yields all its CO_2 when exposed to low pressures.

This shows that factors within the corpuscles must operate in the retention and release of CO_2 . The mechanism has been elucidated, chiefly by the pioneer work of Meldrum and Roughton. Six stages are recognised:—

(1) Dissolved CO_2 passes from tissue fluids to plasma, and enters the red cells.

(2) Within the cell, some CO_2 is bound by combining with the free amino groups of the haemoglobin; the rest is hydrated to H_2CO_3 by means of the enzyme carbonic anhydrase.



(3) H_2CO_3 ionises to HCO'_3 and H^+ , which is buffered by combining with the hæmoglobin.

(4) Some HCO'_3 diffuses out of the cells into the plasma, and is replaced by its equivalent of Cl' , to maintain the anion-cation balance. This "chloride shift" effect is the means whereby the red cells build up HCO'_3 in the plasma.

(5) When the blood reaches the lungs, O_2 enters the red cells, converts the Hb to HbO_2 , and displaces the H^+ . This free H^+ combines with HCO'_3 within the cell, to form H_2CO_3 , which is then converted by carbonic anhydrase to H_2O and CO_2 .

(6) The free CO_2 escapes from the cells into the plasma, and is excreted by the lungs.

The presence of K^+ in the corpuscles, and Na^+ in the plasma, is not shown, as these ionic species are only concerned in the maintenance of anion-cation balance, and not CO_2 transport. For simplicity, also, the formation of carbamino derivatives by union between the CO_2 and the cell and plasma proteins is omitted, although they also aid in carrying CO_2 .

In the circulating blood, most of the carbon dioxide is carried as the HCO'_3 ions of the plasma, which also function as a buffer system for maintaining blood pH by converting H^+ into H_2CO_3 . The red cells operate a mechanism for removing CO_2 from tissue fluids and converting it into plasma HCO'_3 ; while in the pulmonary circulation they remove HCO'_3 from the plasma and convert it into CO_2 for excretion by the lungs.

Buffer Capacity of Blood.—The pH of the entire organism is kept within proper limits by the buffer action of the blood which, in turn, is maintained by the lungs, which remove CO_2 , and the kidneys, which excrete the cations H^+ and NH_4^+ and the anions OH' and HPO''_4 , in accordance with the state of the plasma.

The chief buffers for H^+ in the blood are the proteins of the cells and plasma, the iso-electric points of which are well on the acid side of neutrality (hæmoglobin, pH 6.8; serum albumin, pH 5.5; serum globulin, pH 4.4). At the pH of blood, these proteins have their terminal amino groups free, and are capable of accepting H^+ . The carbonate buffer system, $\text{HCO}'_3 + \text{H}^+ \rightleftharpoons \text{H}_2\text{CO}_3$, and the phosphate buffer system, $\text{HPO}_4 + \text{H}^+ \rightleftharpoons \text{H}_2\text{PO}'_4$, are not working at their optimum range, but provide emergency systems that protect against violent or prolonged strains.

Plasma Alkaline Reserve.—According to Van Slyke, this is defined as "the total volume per cent. of CO_2 liberated when an acid acts on plasma previously brought into equilibrium with a gas mixture containing CO_2 at a partial-pressure of that in alveolar air (40 mm. Hg)." It is an index of the reserve buffer capacity and ability of the plasma to protect the organism against *acidæmia*, or fall in pH below 7.3. The normal range for the alkaline reserve is 53 to 75 ml. CO_2 per 100 ml. Values below 59 denote *acidæmia*; values above 75 denote *hyperalkalæmia*.

The rate of general, or external respiration is governed by the CO_2 ,

output of the tissues, which, by increasing the H^+ and CO_2 concentration in the plasma above the normal level, stimulates the respiratory centre in the pons and upper medulla. For this reason, CO_2 is used to supplement O_2 administration in treatment of carbon monoxide poisoning, and other forms of asphyxia (Yandell Henderson, 1938).

THE PLASMA COLLOIDS

Plasma contains 6 to 8 per cent. of colloids out of a total 8.5 to 10 per cent. of solutes.

Plasma Proteins

- | | |
|---|---------------|
| 1. <i>Serum albumin</i> , m.w. 68,000 | 4.5 per cent. |
| 2. <i>Serum globulin</i> , m.w. 150,000, resolvable by electrophoresis into α -, β - and γ -fractions | 2.2 " |
| 3. <i>Fibrinogen</i> , a globulin | 0.3 " |
| 4. <i>Seromucoid</i> | 0.1 " |

These values are got when the proteins are fractionated by salting-out, a process that may have led to the disintegration of what is really a single protein complex in the original plasma. *Fibrinogen* is precipitated by saturation with NaCl, or 1/5 saturation with $(NH_4)_2SO_4$. *Serum globulins* are precipitated by 1/2 saturation with $(NH_4)_2SO_4$, a variety, *serum euglobulin*, being precipitated by 1/3 saturation. *Serum albumin* requires full saturation with $(NH_4)_2SO_4$ for precipitation. *Seromucoid* is precipitated by acetic acid under special conditions (Howe, 1937-39).

Functions of the Plasma Proteins.—Albumin and, to a lesser extent, the globulins, confer on plasma its colloidal osmotic pressure of 23-28 mm. Hg, or about 0.03 atms. Although this value is small compared with the total osmotic pressure of the blood and tissue fluids (6.9-7 atms.), it is essential for the retention of water within the vascular system and for the maintenance of blood volume.

The capillary walls are permeable to Na^+ and Cl^- , the chief ions of the blood, and when the plasma proteins are depleted by hæmorrhage, renal disease, or starvation, saline fluid escapes into the tissues and accumulates, resulting in local cedema or general dropsy. For this reason, fall in blood volume after severe hæmorrhage cannot be restored for long by injections of isotonic saline. A suitable colloid must be included to enable the transfusate to remain in the vessels. Human plasma is the most effective for this purpose. Blood is collected from healthy donors, treated with an anti-coagulant, and centrifuged free from corpuscles. The resulting plasma is diluted with an equal volume of isotonic saline containing 0.01 per cent. of mercury thiosalicylate as antiseptic, and stored at 5° C., till required. Alternatively, the plasma may be dried to a powder by spraying *in vacuo* at 45° C.

Blood Coagulation.—Within ten minutes of being withdrawn, blood spontaneously thickens and forms a characteristic clot of fibrin strands in which the corpuscles are entangled. Subsequently the clot shrinks and separates from the liquid serum, or defibrinated

plasma. Clotting is a very complex process that involves at least four different factors : (1) *fibrinogen*, the soluble precursor of fibrin ; (2) *prothrombin*, the precursor of the enzyme *thrombin* that converts fibrinogen into fibrin, and requires (3) *calcium ions*, and (4) *prothrombin activators*.

Prothrombin (prothrombase) can be separated from the globulin fraction of the plasma. It is a protein, and is made in the liver by a process requiring vitamin K. The prothrombin level in plasma, normally about 0.04 per cent., is an index of coagulation efficiency. Hypoprothrombinæmia may come from liver injuries, or lack of vitamin K, owing to faulty diet or defective absorption of fats and fat-soluble vitamins. In Quick's method (1938) for estimating prothrombin, Ca^{++} and thrombokinase are added to decalcified blood, and the coagulation time is measured.

Thrombin does not occur to any significant extent in circulating blood, but can be prepared by extracting blood clots with 2N NaCl, and precipitating the enzyme by alcohol.

Lyons (1945) has resolved thrombin into two factors : thrombin A, which exposes thiol groups in fibrinogen, forming fibrinogen B, [$F\text{-SH}$]; this is converted into the insoluble fibrin gel, [$F\text{-S-S-F}$]_n, by thrombin B, which is a naphthoquinone-type of compound, probably derived from vitamin K.

Calcium ions are necessary for the conversion of prothrombin into thrombin, and when removed by precipitation with fluoride or oxalate, or by addition of citrate, which forms a non-ionised Ca complex, the resulting decalcified blood or plasma will no longer clot, unless thrombin be added. Decalcification is the simplest method for preventing the coagulation of blood required for storage, transport or analysis. It can be effected by adding a little powdered potassium fluoride or citrate to the tubes in which the blood will be collected.

Prothrombin Activators.—*Thrombokinase* (thromboplastin) occurs in tissues and in the blood platelets, from which it separates out as a granular precipitate that forms when oxalated blood is kept at 0° C. for some days. It is a lipoprotein in which the phospholipide cephalin is the prosthetic group. Other agents capable of activating prothrombin are enzymes of the trypsin type, including pure crystallised trypsin, and one of these occurs in blood as serum tryptase. According to Ferguson (1943), tryptase is the chief factor in activating prothrombin, while Howell claims that three different thrombokinases exist, derived, respectively, from plasma, platelets and damaged tissues.

Coagulation Inhibitors.—Blood can be defibrinated by being stirred rapidly by a bundle of fine wires, which collects the fibrin as

it forms and leaves a suspension of corpuscles in plasma. Coagulation of blood in bulk can be retarded by low temperature, freedom from agitation and absence of rough surfaces, thus repressing the release of prothrombin activators.

In the circulation, clotting is prevented both by the absence of the necessary factors, thrombokinases and thrombin, and also by the presence of special inhibitors that retard the formation of thrombin and fibrin.

Heparin, a coagulation inhibitor discovered by Howell (1918) in liver and lung extracts and later obtained from blood basophil leucocytes. According to Jorpes (1939), heparin is an ester of mucoitin sulphate or chondroitin sulphate, and coacts with a factor present in normal plasma to yield a powerful anti-coagulant. Heparins from different species differ in potency. Representative values, expressed in heparin units per mg., are : dog, 240 ; ox, 100 ; pig, 44 ; 100 units being defined as the amount of heparin that can prevent clotting in 10 to 20 ml. of blood at ordinary temperatures.

Like other complex esters of sulphuric acid, heparin is precipitated and stained by dyes of the toluidin blue class, staining being accompanied by a metachromatic change of the dye from blue to red.

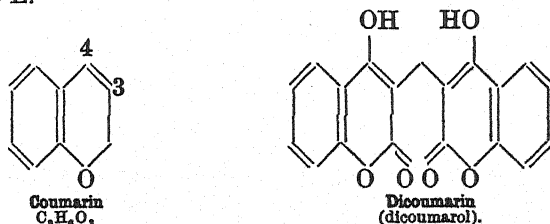
The basophil, or "mast" cells that are distributed along the capillary bed, and circulate in the blood, stain metachromatically with toluidin blue. Whenever the heparin content of the blood is increased, as in response to anaphylactic shock or peptone injection, these cells lose their stain-fixing granules, which indicates that the basophils are the source of the heparin.

Anti-trypsin.—According to Volkert (1942), only a small and variable part of the anti-coagulant power of plasma is due to heparin ; about 80 per cent. is constant, and is related to the protein content, and may be a true anti-trypsin (Ferguson, 1942).

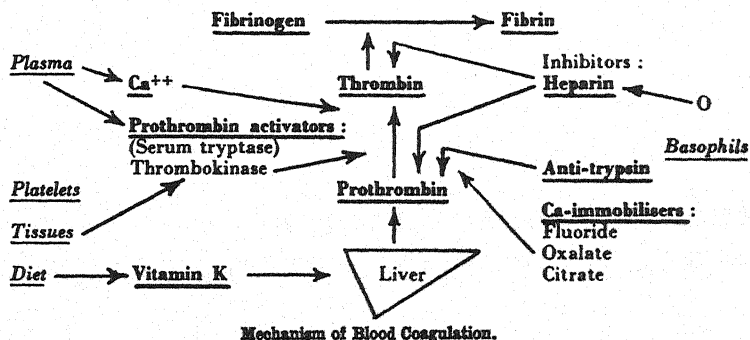
Hirudin, a glycoprotein from the oral secretion of the leech, *Hirudo medicinalis*, is representative of the anti-coagulants secreted by various blood-sucking animals to protect themselves from being choked by blood clots. These factors must have been elaborated at a stage later than the evolution of the mammalian blood-coagulation mechanism, and they provide an interesting example of biological adaptation and counter ingenuity.

Dicoumarin.—A disease characterised by delayed clotting time and a tendency to hæmorrhage can arise in cattle that have eaten sweet clover "spoiled" by infection. The agent has been isolated and identified (Link *et al.*, 1940). It is a dicoumarin formed from two molecules of 4-hydroxy coumarin united by a $-\text{CH}_2-$ bridge between the carbons at position 3. The therapeutic applications of this 3:3'-methylene-*bis* (4-hydroxycoumarin) in treatment of

thrombosis have been investigated. The parent compound, coumarin, is present in many plants, and imparts the characteristic odour to freshly cut grass. Its ring system is part of the structure of vitamins E.



Artificial anti-coagulants of the poly-sulphate ester type have been prepared, including "disodium cellulose disulphate" and "Chicago blue." Chondroitin monosulphate, which occurs free in cartilage, and stains metachromatically with "toluidine blue," only acquires anti-coagulant properties after it has been esterified by an additional $-O.SO_2.OH$ group.



THE BIOCHEMISTRY OF THE ERYTHROCYTE

The adult human body contains 600 to 700 gm. of hæmoglobin, circulating in the 30×10^{12} red cells of the blood. Hæmoglobin is elaborated in the red marrow from pyrrole rings supplied by the tryptophane of the diet. Each tetrapyrrole cluster thus assembled unites with an iron atom, derived partly from the diet and chiefly from disintegrated red cells, forming a ferrous porphyrin, or hæm pigment. The process is controlled by a Cu-containing catalyst and ascorbic acid. While this is taking place, the primitive red cell has been developed from the endothelial cells of the red marrow sinusoids as a *pro-normoblast*, or *pseudo-megaloblast* in which the stroma of the red cell is formed through the agency of a maturation factor supplied by the liver.

Pigmentation of the developing cell now occurs, producing an

erythroblast, in which the hæm has been combined with globin. By loss of the nucleus, an immature erythrocyte, or *reticulocyte*, is formed, and eventually issued into the general circulation as a mature red cell.

Histological evidence is got by sternal puncture and aspiration of samples of living red marrow, which show the changes due to depletion by hæmorrhage or starvation, and stimulation by hæmatinic agents or environments poor in oxygen. In aplastic anæmias, cell-formation is checked at the pre-megaloblastic stage; in pernicious anæmia, due to lack of the specific maturation factor, cell development is checked at the megaloblast stage, and the megaloblast population in sternal marrow may rise from the normal range of 25,000–35,000 cells per cu. mm., and reach levels of 60,000–120,000. When iron, copper or ascorbic acid is deficient, a nutritional hypochromic anæmia may develop, in which the cells have not their full load of hæmoglobin.

Life-span of the Erythrocytes.—After having been in circulation for, apparently, two to four weeks, the red cells are trapped by the reticulo-endothelial tissue of the spleen, liver and bone marrow, and are disintegrated. The iron is released, and most of it is mobilised in the red marrow, and is taken up by the developing erythrocytes and reformed into hæmoglobin. The rest is stored as a protein, *ferritin*, and as a ferric complex, *hæmosiderin*, chiefly in liver and kidney tissue. Iron is absorbed from the diet in the form of Fe^{++} , the process being regulated in accordance with the hæmoglobin need of the organism.

After absorption, iron is not excreted to any significant extent in the urine or secretions of the intestine, apart from a little in the bile, and the store of the metal in the organism increases with the years.

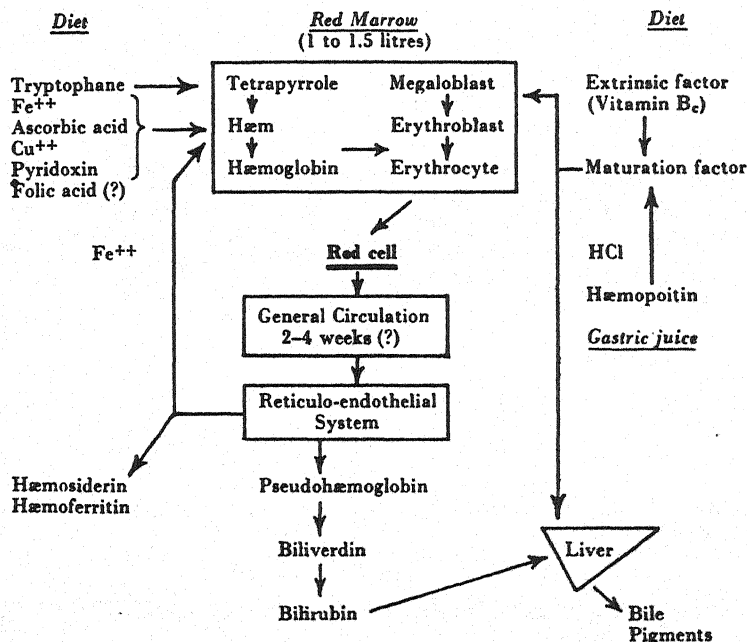
In the pathological condition of *hæmatochromatosis*, fixation of iron in the tissues is greatly increased, yet there is no evidence of increased rate of red cell destruction (Sheldon, 1935).

On disintegration of the red cells, hæmoglobin escapes, and is degraded to "pseudohæmoglobin," or verdohæmoglobin, in which the pyrrole ring is opened up into a linear tetrapyrrole. By loss of globin and Fe, this forms biliverdin.

According to Lemberg, hæmoglobin degradation may begin in the ageing red cell before disintegration, and thus may determine the life-span of the erythrocyte. It is possible, though hard to establish, that some pseudohæmoglobin escapes conversion into bile pigment, and is mobilised in the red marrow for the manufacture of hæmoglobin.

Estimates of the life-span of the adult erythrocyte in man range from four to 120 days, according to the method of assessment.

The average period, generally accepted, is two to four weeks, and is based on the time of survival after injection of a known amount of isotopically labelled red cells.



The Biochemistry of the Erythrocyte.

AVERAGE COMPOSITION OF HUMAN BLOOD

Major constituents, expressed as percentage

	Blood, Entire.	Plasma, or Serum.
Solids, total	18-25	8.5-10
Corpuscles, volume	36-51	—
Proteins, total	17.8-24.6	5.8-8.6
Hæmoglobin (men)	13-17.5	0
(women)	12-14	0
Orosins (F + A + G) :	—	5.8-8.6
Fibrinogen (F)	0.1-0.2	0.2-0.4
Albumin (A)	—	3.4-6.7 (average, 4)
Globulin (G)	—	1.2-2.9 (average, 2)
A : G ratio	—	4 : 1-1.2 : 1 (average, 2 : 1)
Lipides, total	0.2-2	0.45-1.2
Fatty acids	0.29-0.42	0.19-0.64
Chloride, as NaCl	0.45-0.53	0.56-0.62
as Cl ⁻	0.28-0.32	0.34-0.38

AVERAGE COMPOSITION OF HUMAN BLOOD

Minor constituents, expressed in mg. per 100 ml.

	Blood, Entire.	Plasma, or Serum.
Nitrogen, non-protein :	25-50	18-30
Amino acid N	3-8	4-6.5
Urea N	7-20	7-20
Creatine N	0.6-2.9	0.2-1
Creatinine N	0.3-0.7	0.3-0.7
Uric acid N	0.1-1.3	0.1-1.3
Residual N	3-19	2-12
Sugar, as glucose	60-180	60-180
Cholesterol, total	100-200	100-220
Bile acids :		
as glycocholic acid	2.5-6.0	—
as Na glycocholate	—	5-12
Bilirubin	—	0.1-0.5
Urobilin	—	0.0-0.4
Glutathione, total	28-522	0.0
Lactic acid	5-35	—
Creatine	2-9	0.5-3
Creatinine (?)	0.7-2	0.7-2
Urea	15-40	15-40
Uric acid	0.3-4	0.3-4
Phenol	2-8	—
Indoxyl sulphate	—	0.03-0.08
Ketones, as acetone	1-3	—
Bromide, as NaBr	—	0.5-2.5
as Br ⁻	0.23-0.57	—
Calcium, total	5-7	9-11
diffusible	—	3.6-4.6
non-diffusible	—	2.8-6.1
Iodine	0.005-0.02	—
Iron	45-55	0.06-0.22
Magnesium	2-4	1-4
Phosphorus, total P	28-48	6-18
Inorganic P	2-5	2-5
Ester P	14-29	0-4
Lipoid	8-18	3-14
Potassium	150-250	18-21
Sodium	170-225	325-350
Sulphur, non-protein :		
Total S	3.8-5.1	3.1-3.9
Inorganic S	0.28-0.65	0.5-1.1
Ester S	0.07-0.96	0.09-0.96

Blood Analysis

Blood analysis has now become an important part of clinical medicine, both in establishing diagnoses and in observing progress of many diseases, of which diabetes and nephritis are the most obvious. By the use of a micro-technique, routine analyses can be carried out on 0.2 ml. samples of material. The methods adopted

depend on laboratory facilities and clinical requirements, and are modified in accordance with the needs of different workers. Established methods are described in the standard text-books, such as :—

Peters and Van Slyke, "Quantitative Clinical Chemistry," Vol. II.

Hawk and Bergeim, "Practical Physiological Chemistry."

Harrison, "Chemical Methods in Clinical Medicine."

Beaumont and Dodds, "Recent Advances in Medicine."

Current analytical procedure is being continually revised and augmented by work published in the scientific and medical journals.

CHAPTER 26

TISSUE CHEMISTRY

Tissues are functional assemblies of cells. They are not structures of fixed composition, but are regions of specialised metabolism and, when integrated into organs and systems, may serve various purposes of secretion, storage and transformation. Hence histochemistry, the chemistry of tissues, is apt to appear as a mere biochemical catalogue of contents, many of which are obscure, unless it is studied along with physiology and microscopic anatomy. Nevertheless, each of the chief tissues has one or more characteristic constituents, which determine its function. Four of these tissues will be considered :—

1. **Muscle**, the motor tissue of the animal, makes up about 40 per cent. of human body weight, and dominates metabolism. Voluntary muscle is the instrument of the will, the only physical means whereby man can bring about changes in his environment. Cardiac muscle, by its inherent rhythm, maintains the internal fountain that supports life and, incidentally, provides us with a natural wrist-watch, the pulse, which may be the basis of our sense of time. Involuntary, or unstriated, muscle produces the necessary stresses and automatic movements within the body. All told, muscle activity accounts for about half of the entire metabolism of the body during rest, and upwards of three-fourths during exertion.

The three varieties of muscle: skeletal (striated), cardiac and visceral (unstriated), differ in composition, even when present in related structures, such as the cardiac ventricles (Myers, 1934). The general percentage composition is: water, 75–80; proteins, 18–20 (myosin, 12·4; globulin x, 4; myogen, 2; myoalbumin, 0·2); lipides, 1–2; glycogen, 0·5–1·5; creatine, 0·16–0·5; inositol, 0·02; K, 0·2–0·33; Na, 0·05–0·15; Ca, 0·03 (striated), 0·007 (unstriated); Fe, 0·004; PO₄, 0·2–0·6; Cl, 0·28 (striated), 0·1 (unstriated); together with small amounts (0·01–0·1) of obscure reactants, such as carnosine, carnitine, anserine, methyl guanidine, hypoxanthine and inosinic acid, some of which may participate in the contraction cycle.

Myosin, the elastic contractile component of the muscle fibre, is an extensible fibrous globulin, the elongated molecules of which form a periodic or repeating pattern, and render the central part of each sarcomere in the fibril optically *anisotropic*, or doubly refracting, when viewed by polarised light. Myosin-extracts in solutions at rest are randomly distributed, and shows no double refraction, but when

the solution is set in motion it becomes anisotropic, showing that the rod-like myosin molecules have been orientated into a pattern. A myosin solution tends to form a gel, which has the property of reverting temporarily to a sol on being shaken vigorously. This *thixotropy*, or reversible gelation, is displayed by some other colloidal systems, and is ascribed to spontaneous recovery of the molecular mesh of the gel, after disarrangement.

Myosin is an enzyme, and catalyses the conversion of adenosine triphosphate, *ATP*, to diphosphate, taking up the energy liberated in the process. This is the first recognised example of a substrate-enzyme reaction accompanied by a mechanical change in the state of the enzyme. It has been made the basis of a theory of muscular contraction (Engelhardt, 1941; Needham *et al.*, 1941). According to Szent-Györgyi (1945), myosin is combined with another protein, *actin*, as a actomyosin, the contractile substance of muscle.

2. Epithelial tissue, in which the intercellular material is minimal, forms the lining of the respiratory and alimentary tracts, and the skin and epidermal structures (hair, nails and horn) of the body surface.

The percentage composition of fresh skin, as compiled by Williams (1942) is: water, 66; proteins (keratin and collagens), 25; lipides, 7 (phospholipides, 0.6; cholesterol, 1); total ash, 0.65 (Na, 0.16; K, 0.09; Cu, 0.01; Mg, 0.007; Fe, 0.001; PO_4 , 0.2; Cl, 0.1, CO_2 , 0.005).

Keratins, the characteristic insoluble scleroproteins of skin and hair, have a high percentage (15–21) of cystine (p. 139).

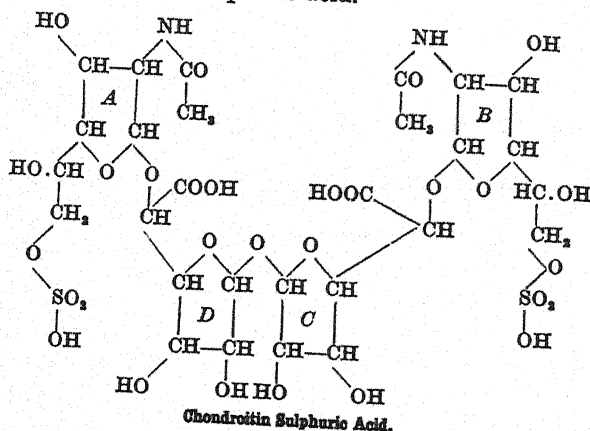
3. Connective tissue, in which the intercellular material, or matrix is maximal, forms the tensile and skeletal fabrics of the animal. Five varieties are recognised, depending on the composition of the intercellular matrix.

White fibrous tissue. Percentage composition: water, 62.9; proteins, 34 (collagen, 31.6; elastin, 1.6); mucoid, 1.2; lipide, 1.0; inorganic residue, 0.5. *Collagen*, the characteristic constituent of white fibres in tendon and elsewhere, is a protein with a high percentage of glycine (25), proline (20) and hydroxyproline (14), but poor in tryptophane (0), valine (0), cystine (0.1) and tyrosine (0.01). It is insoluble in water, but may be hydrolysed by prolonged boiling, when it is converted into *gelatin*, which is soluble.

Yellow elastic tissue. Percentage composition: water, 57.6; proteins, 40 (elastin, 31.7; collagen, 7.2), lipides, 1.1; mucoid, 0.5; inorganic residue, 0.5. *Elastin*, the characteristic insoluble protein of yellow elastic tissue, well represented in the *ligamentum nuchae* of the ox, like collagen has a high percentage of glycine (30), and proline (15), and is poor in tryptophane, cystine and tyrosine.

It differs from collagen in having a high percentage of valine (13.5) and leucines (30), as shown by Stein and Miller (1938). Unlike collagen, elastin is not digested by gastric juice, and does not form gelatin when hydrolysed.

Cartilage, the covering material of articular surfaces, and the precursor of bone, has a matrix containing about 30 per cent. of a characteristic glycoprotein, *chondromucoid*, which on hydrolysis liberates *chondroitin sulphate*, a complex mucopolysaccharide assembled from two molecules of gluconic acid and two molecules of N-acetyl galactosamine, each terminal, or 6 hydroxyl group of which is esterified with sulphuric acid.



A and *B* are galactosamine residues in which the amino group has been acetylated, and the terminal hydroxyl at 6 has been esterified with H_2SO_4 .
C and *D* are glucuronic acid residues, united by ether linkage.

Small amounts of chondroalbuminoid, a scleroprotein resembling elastin, also occur in cartilage.

Salivary mucin contains *mucoitin sulphate*, the structure plan of which is the same as chondroitin sulphate, but for the hexosamine residues, which are glucosamine instead of galactosamine. These complex mucopolysaccharides are of interest in being components of the heparins that inhibit blood coagulation.

In the connective tissues, chondroitin sulphate exists as a polymer, of *M.W.* 200,000.

Hyaluronic acid, formed by removal of both sulphate groups from mucoitin sulphate, is the prosthetic group in mucoproteins. It is hydrolysed by the enzyme hyaluronidase, secreted as a diffusing factor by bacteria.

Bone.—The skeletal tissue of man and higher animals is a cartilaginous matrix stabilised by deposition of lime salts in a characteristic pattern.

Composition.—Intact bone contains 23 to 34 per cent. of water and up to 12 per cent. of marrow fat. Dried, marrow-free bone contains about 60 per cent. of inorganic salts, which may be dissolved by prolonged extraction with HCl ; and 40 per cent. of organic material, which may be removed by incineration, leaving bone ash. This organic

material is chiefly *ossein*, a bone protein closely resembling collagen, and, like it, yielding gelatin when bones are digested with boiling water. Ossein is accompanied by small amounts of *bone keratin*, *osseoalbuminoid* and *osseomucoid*, which resembles the chondromucoid of cartilage.

The Bone Salts.—The percentage composition of bone ash is : calcium phosphate, $\text{Ca}_3(\text{PO}_4)_2$, 85 ; calcium carbonate, CaCO_3 , 12 ; magnesium phosphate $\text{Mg}_3(\text{PO}_4)_2$, 1.4 ; with a residue of salts of Na(0.4), K(0.13), and traces of Fe, Cl and F. Fresh bone contains 0.2–0.3 per cent. of citrate, which reappears as carbonate in bone ash. Bone salt composition varies with region and age, and cannot be referred to any one single mineral type. Chemical analysis and crystalline structure, as shown by X-rays, suggest that bone calcium is present as the double salt, $3\text{Ca}_3(\text{PO}_4)_2 \cdot \text{CaCO}_3$, resembling that found naturally in minerals of the *apatite* series, $[\text{Ca}_3(\text{PO}_4)_2]_n \cdot \text{CaCO}_3$, where n has a value between 2 and 3, and the CaCO_3 may be replaced by $\text{Ca}(\text{OH})_2$, CaSO_4 , CaCl_2 or CaF_2 . In mammalian bone, $n = 3$, and the Ca : P ratio is almost constant between 1.99 and 2.04, which is less than the common apatite ratio of 2.15, and may be due to replacement of some 6 per cent. of the Ca by Mg, Na or K.

Decrease in the value for n , resulting in "high carbonate" bone, occurs in old age, in rickets due to phosphate insufficiency, and in the pathological condition of "marble bone."

Calcification.—Bone formation is a process in which calcium salts are laid down in a protein matrix. The salts are not precipitated as a more or less homogeneous sediment, but are systematically assembled in a pattern conforming to the physical requirements of growth, stress and repair. The exact mechanism of the process is not yet clear. Bone salt is derived chiefly from three species of ions present in the blood plasma : Ca^{++} , $\text{H}_2\text{PO}_4'$ and HCO_3' . In accordance with the solubility rule, calcium phosphate and calcium carbonate are precipitated from aqueous solutions whenever the concentrations of the component ions exceed the values for the respective solubility products.

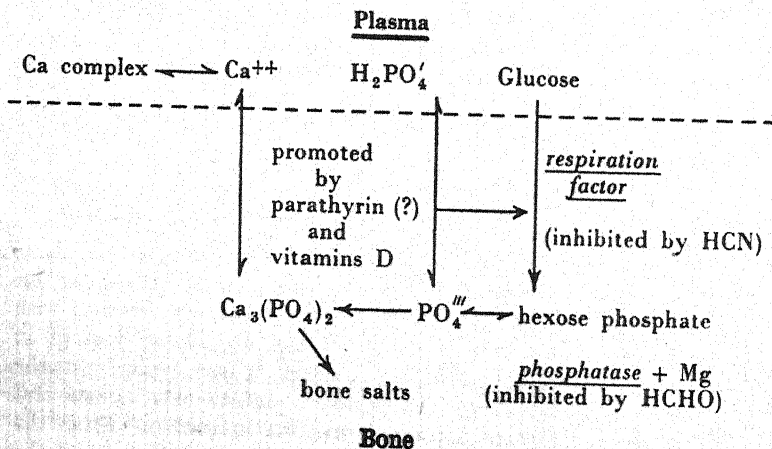
The conditions for precipitation at 38° C. are :—

$$[\text{Ca}^{++}]_2 \times [\text{PO}_4']_2 > 10^{-32.5}, \text{ and } [\text{Ca}^{++}] \times [\text{CO}_3'] > 1.7 \times 10^{-8}.$$

Since calcium precipitation does not occur normally in the circulating blood, the solubility product value is not exceeded ; or, alternatively, plasma may be in a state of supersaturation with regard to the bone salts. Of the 10 mg. of calcium present in 100 ml. of plasma, only 2 to 3 mg. occur in free, ionic form, the remainder being bound by the proteins, amino acids, citrate and other factors capable of depressing calcium ionisation. From the graph on p. 57, it will be seen that, at the pH of blood most of the

phosphate and carbonate are present as $\text{H}_2\text{PO}_4'$ and HCO_3' , the concentration of the ions PO_4''' and CO_3'' being almost zero. Hence, for calcium salts to be deposited in growing bone, some local mechanism must operate to raise the concentration of PO_4''' and CO_3'' . In 1923, Robison found that bone is very rich in a phosphatase of the alkaline type (optimal pH, 8-9), and he was able to bring about calcification in strips of rachitic bone by immersing them in solutions of Ca^{++} and glycerophosphate or hexose monophosphate.

According to Robison's original theory, calcification is due to the localised liberation of PO_4''' from monophosphoric esters of the plasma, by the action of the "bone enzyme" secreted by the osteoblasts. The chief objections to this theory are: (1) non-calcifying tissues, such as kidney, intestine and leucocytes, may be rich in phosphatases of the alkaline type; (2) in rachitic conditions, where calcification is defective, the phosphatase present in the bones and the blood plasma is greatly increased, and may provide a means for diagnosing the disease; (3) bone phosphatase only attacks monophosphoric esters, but in the blood these esters are almost entirely restricted to the red cells. In 1926, Shipley observed that calcification of bone strips can occur when they are immersed in serum or in sterile salines of the same ionic composition, showing that preformed phosphoric esters are not necessary. Under these conditions, however, it was found that cyanide, iodoacetate or fluoride had an inhibitory effect not displayed in Robison's original experiments. As these agents all retard glycolysis, it is now believed that calcification proceeds by a metabolic sequence in-



Mechanism of Calcification.

volving: (a) carbohydrate degradation, with formation of phosphoric esters, and consequent accumulation of phosphate from the plasma, and (b) hydrolysis of the esters by the bone phosphatase. Glycogen, which is present in small amounts in growing bone and teeth, may represent a by-product of this calcification metabolism.

Teeth.—A tooth is a highly specialised unit consisting of three calcified parts: (1) an enamel layer of epidermal origin, which is the hardest tissue in the body; (2) the dentin, which is of mesodermal origin, and forms the main substance of the tooth, and encloses the pulp cavity containing the blood vessels and nerves; (3) the cementum, which fixes the root dentin in its socket in the alveolar bone, supported by the gum, or gingival tissue, and the periodontal membrane.

Percentage Composition of Teeth
(Bowes and Murray, 1935)

	Enamel.	Dentin (dry).
Ash . .	95.38	71.09
Nitrogen . .	0.156	3.43
H ₂ O, bound .	1.347	
CO ₂ , bound .	1.952	
Ca . .	37.07	27.79
Mg . .	0.464	0.83
Na . .	0.25, or less	0.19
K . .	0.05	0.07, or less
P, . .	17.22	13.81
(as PO ₄) . .	(51.6)	(44.4)
Cl . .	0.3	0.000
F . .	0.025	0.024
Si . .	0.003 { for entire tooth	
Ca/P . .	2.153	2.012 { (Ca/P for apatite, 2.151)
Ca/Mg	78.89	33.29

A study of the composition of enamel, dentin and dental pulp has been made by Fish (1932), and the analytical results of different investigators have been summarised by Armstrong (1942).

Factors in the Growth and Maintenance of the Teeth.—M. Mellanby (1929-34) has shown that dietary factors similar to those concerned in bone calcification operate in the development of the teeth. The erupted tooth, however, differs from bone in that it is continually

exposed to abrasion and chemical attack from bacterial products formed in the mouth. Surface abrasion is resisted by the enamel layer, the integrity of which requires the presence of fluoride as a micro-constituent. The halogen is derived principally from the local water supplies, its optimal concentration being 0.5 to 1 part per million. Values below 0.2 p.p.m. predispose to faulty enamel formation, and promote dental caries; values above 5 p.p.m. may lead to chronic *fluorosis*, one of the signs of which is rough, mottled and brittle enamel. Claims have been made of the value of minute doses of fluoride or bone powder in the prevention of caries, but precautions must be taken to avoid overdosage. In addition to vitamins D and the parathyroid hormone, which regulate the Ca and PO_4 distribution, vitamin A, according to M. Mellanby, is required for the maintenance of the sub-gingival epithelium in contact with the enamel, and vitamin C is necessary for the maintenance of the *odontoblasts*, which cover the pulp, and penetrate into the tubules of the dentin.

The biochemistry of dental decay has been reviewed by Marshall (1939), Gies (1941) and Armstrong (1942). In addition to the consequences of faulty construction, due to inadequate maternal or post-natal diet, factors that may be implicated are (1) saliva deficient in buffering action, and (2) local infection of the mouth by acid-forming organisms, such as *L. acidophilus*, fed by excess of sugars in the diet.

Brain and Nerve.—Two types of material occur in the nervous system: (a) *white substance*, forming the sheaths of the medullated nerves; and (b) *grey substance*, which is well-represented in the cortical layers of the brain, and consists of nerve cells and their connections. The pioneer work of Thudichum (1884) has shown that both white and grey substance are characterised by a high content of cholesterol and complex lipides, which is not depleted by starvation, and must have functional significance.

Composition.—According to the data collected by Williams (1942) and others, entire fresh brain has the percentage composition: water, 78; proteins, 8; phospholipides, 6; cerebrosides, 3; cholesterol, 2; sulpholipides, 1; glycogen, 0.09; glucose, 0.01; total ash, 0.8; K, 0.15; Na, 0.08; Ca, 0.01; Mg, 0.01; Cl, 0.13; Fe, 0.008; Cu, 0.0025; Ni and Co, traces; ascorbic acid, 0.025 (cerebellum), 0.015 (cerebrum); creatine, 0.2; glutathione, 0.1; inositol, 0.2; lactate, 0.1; choline and acetyl choline, 0.002.

Palladin and his colleagues (1935-36), during a survey of the chemical topography of the nervous system, have reported the following values:—

Percentage Composition of Fresh Nerve Tissue

	Spinal Cord, Grey Matter. Dog.	Medulla, Grey Matter. Dog.	Cerebral Cortex. Dog.	Sym- pathetic Trunk. Cow.
Water	71.6	81.6	80.7	59.3
Proteins	9.9	9.8	10.1	22.5
Phospholipides, unsaturated	6.1	2.9	2.7	1.5
Cerebrosides, and saturated phospho- lipides	4.4	3.1	2.8	—
Cholesterol	4.3	1.8	1.5	1.3

The nerve proteins are chiefly nucleoprotein and globulin, with a small amount of *neurokeratin*, associated mostly with the fibres.

The unsaturated phospholipides are chiefly lecithin, which is a source of the choline used in assembling the acetyl choline necessary for the transmission of nerve impulses. Lipides containing a neuraminic acid derived from neurine, occur in nerve ganglia, and are termed *gangliosides* (Klenk, 1942).

Transmission of a nerve impulse is accompanied by uptake of oxygen, and release of carbon dioxide, free phosphate, and traces of ammonia from an unidentified precursor. Even when at rest, a nerve absorbs oxygen; the Q_{O_2} for rabbit nerve at 37° C. being about -1.15, a value increased nearly four times by stimulation. Nerve can go into a state of "oxygen debt," as shown by its ability to continue to conduct for some hours, when in an atmosphere of nitrogen. Under these anaerobic conditions, lactate is formed from carbohydrate; but there is insufficient evidence to show that lactate is a normal intermediate in aerobic conduction. Brain tissue, as already explained in Chapter 14, uses glucose as its chief, if not exclusive, source of energy, and appears to oxidise it completely, by way of pyruvic acid. The small amount of glycogen present in brain is insufficient to provide for the carbohydrate needs of the tissue, which must be immediately dependent on the cerebro-spinal fluid for its oxygen and glucose supplies.

Cerebro-spinal fluid is a colourless filtrate from blood plasma. Normally, it is clear, and free from cells, apart from 1-5 lymphocytes per cu. mm. The total volume in a healthy adult is about 130 ml. The pH is 7.35-7.4, and the sp. g. is 1.006-1.008.

Composition, in mg. per 100 ml.: total protein, 10-35 (average values, albumin, 20; globulin, 3); glucose, 45-100; Na^+ , 325; Cl^- , 425 ($NaCl$, 700-760); $H_2PO_4^-$, 1.25-2.0; K^+ , 12-17; Mg^{++} , 3; Ca^{++} , 4-7; urea, 10-40; lactate, 8-26; amino acid nitrogen, 1.5-3; creatinine, 1.5; uric acid, 0.5-2.8 (?); cholesterol, 0.00; glutathione, 5; citrate, 0.3; CO_2 , 40-60 ml. per 100 ml.; O_2 , 0.1-0.3 ml. per 100 ml.

Data such as these show that of all the constituents, only Na and Cl are present in concentrations higher than those of the blood plasma. Proteins are increased in inflammatory conditions of the meninges, and in general paralysis, tabes and some other syphilitic states. This may be shown by a positive *Nonne-Apel* reaction for globulin, in which the excess protein appears as a cloudy intermediate zone when 1 ml. of cerebro-spinal fluid is layered on 1 ml. of saturated ammonium sulphate solution.

Significance of the Nervous System.—Nerve tissue has two dominant functions : (1) as an instrument of purpose, it co-ordinates and centralises the activities of the entire animal ; (2) as an instrument of learning, it is the only tissue capable of being trained, and showing the property of recording and remembering past experience. When the brain has reached a certain level of complexity it controls behaviour, and its activity is referable to an entity, the *mind*. In its higher manifestations, mind reveals itself as : (1) an organisation of previous experiences that makes possible the rationalisation of present experiences, and the prediction of future experiences ; together with (2) a continual impetus to act, rationalise and predict. The central nervous system provides a well-protected and retentive tissue structure in which the association tracts and patterns that form the memory can be woven by the aid of the intense metabolic activity that characterises the respiration of the brain.

AUTOLYSIS

" There was a purpose in all earthly things ;
And it is gone, and they asunder fall."

DUNSANY.

Cells have an inherent property of self-construction that enables them to grow and to preserve their character. Under certain conditions the process is reversed, and the cells and tissues undergo *autolysis*, or self-digestion, as is shown by the breakdown of the proteins of the cytoplasm and other cell constituents. Localised autolysis is a normal event during the life-cycle of many animals, and is seen in the atrophy of the uterus and mammary glands, after pregnancy and the lactation period.

A more widespread autolysis occurs during starvation, when there is a selective mobilisation of tissue proteins to provide the amino acids necessary to maintain life. Tissues of less survival value are attacked first, the order being : liver, skeletal muscle and, finally, heart and respiratory muscle. The osseous, cartilaginous, elastic and fibrous tissues which hold the organism together undergo little or no autolysis. The central nervous system appears to be completely resistant.

While the organism is alive, autolysis is a reversible process, and depends on equilibrium maintenance between the products of cell metabolism and the constituents of the internal environment. Thus, muscular atrophy, following immobilisation or nerve injury, may result in loss of half of the total muscle substance, without destruction of the muscle cells. The contraction mechanism persists, and the muscle can regenerate completely as soon as circumstances become favourable.

Immediately after the death of the organism, however, a general post-mortem autolysis sets in, and brings about the softening and liquefaction of the tissues.

The process is evoked by lack of available oxygen and by increase in acidity, and depends mostly on the release of protein-splitting enzymes of the cathepsin type, within the cells.

The reaction of the living cell, pH 6.8–7.2 (Chambers and Kerr, 1932), is unfavourable for autolysis. The process is detectable about pH 6, reaches a maximum at pH 4, and then falls to zero, when the acidity is raised to pH 2.5–2, in which region the enzymes are destroyed.

Post-mortem liberation of acid is independent of protein breakdown, and probably is due to failure of the tissue respiration systems. Autolysis is accelerated by mechanical disintegration of the tissues, and can be brought about in yeasts and bacteria by agents, such as ether or acetone, that damage the cell surface. The progress of the change can be followed by incubating suspensions of finely disintegrated tissue in presence of an antiseptic, such as toluene or salicylic acid, that does not precipitate the proteins or inactivate the enzymes.

Samples are analysed by precipitating the unchanged protein, and estimating the amino acids and peptides in the filtrate.

Autolysis reaches equilibrium within 5 to 10 days, depending on the temperature and the type of tissue. It is inhibited by oxidising agents, such as iodine and iodate, and is promoted by reducing agents, including cysteine and other thiols, which activate enzymes of the cathepsin type.

Autolysis is a process quite independent of putrefaction or other infective changes, and Bradley (1938), who has studied it extensively, regards it as a manifestation of the most fundamental and primitive enzyme mechanism residing in all cells.

*"When that which drew from out the boundless deep
Turns again home."*

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APPENDIX I
Representative Food Materials
Composition per 100 gm.

<i>Foodstuff, 100 gm. (3½ oz.).</i>	<i>Energy, in kilocal.</i>	<i>Water, in gm.</i>	<i>Protein, in gm.</i>	<i>Fat and other lipides, in gm.</i>	<i>Carbohydrate, available, in gm.</i>	<i>Phosphate, as P, in mg.</i>	<i>Calcium, in mg.</i>	<i>Iron, in mg.</i>
<i>Animal products :</i>								
Bacon (raw) . . .	500	30	10	40	0	115	13	1.2
Beef, lean (raw) . . .	117	68	14	10	0	276	5	4.3
Brain, sheep (boiled) . . .	110	79	12	7	0	339	11	2.2
Chicken (boiled) . . .	203	61	26	10	0	270	10	2.1
Ham (raw) . . .	517	31	15	49	0	104	14	1.2
Kidney, sheep (raw) . . .	98	77	17	3	0	254	13	11.7
Liver, ox (raw) . . .	143	73	16	8	tr.	313	8	14
Mutton (boiled) . . .	260	45	12	16	0	238	4	5.4
Pork (roast) . . .	317	50	24	23	0	363	5	1.7
Rabbit (stewed) . . .	180	64	26	8	0	119	11	1.9
Tongue, ox (boiled) . . .	310	49	19	2	0	229	31	3
Veal (raw) . . .	108	56	13	5	0	258	7	2.3
Egg, hen (raw or boiled) . . .	163	73	12	12	0	218	56	2.5
Egg, white . . .	37	88	9	tr.	0	33	5	0.1
Egg, yolk . . .	350	51	16	30	0	495	131	6.1
Milk, cow . . .	66	87	3.3	3.6	4.7	100	120	0.03
Milk, powdered . . .	485	5	25.6	26.7	35.6	712	900	1.6
Milk, skimmed . . .	36	90	3.4	0.2	4.8	98	124	0.08
Butter . . .	745	15	0.5	82	tr.	24	15	0.16
Cream . . .	407	53	1.8	42	2.3	25	59	0.23
Cheese, Cheddar . . .	423	37	25	34	tr.	545	810	0.57
<i>Fish :</i>								
Cod (steamed) . . .	82	79	18	1.9	0	242	15	0.5
Herring (raw) . . .	140	50	11	10.5	0	272	70	1.5
Lobster (boiled) . . .	119	72	21	3.4	0	283	62	0.8
Mackerel (fried) . . .	187	66	20	11	0	280	28	1.2
Plaice (steamed) . . .	92	42	18	2.9	0	246	38	0.6
Prawn (cooked) . . .	104	70	21	2.8	0	349	145	1.1
Salmon (steamed) . . .	199	65	19	13	0	302	30	0.8
Sardine (canned) . . .	294	51	20	23	0	683	409	4
Sole (steamed) . . .	84	47	18	1.3	0	270	113	0.7
<i>Cereal products :</i>								
Barley, pearl . . .	384	10.6	8.4	1.7	81.3	206	8	0.67
Cornflour . . .	387	11.5	0.6	0.7	92	39	15	1.43
Macaroni . . .	383	10.4	11.7	2.0	77	152	26	1.43
Oatmeal . . .	434	3.5	13.3	8.7	73	380	55	4.12
Rice, polished . . .	393	11.7	6.8	1.0	87	99	4	45.0

<i>Foodstuff, 100 gm. (3½ oz.).</i>	Energy, in kilocal.	Water, in gm.	Protein, in gm.	Fat and other lipides, in gm.	Carbohydrate, available, in gm.	Phosphate, as P, in mg.	Calcium, in mg.	Iron, in mg.
Wheat, entire (100%).	317	13	8-15	2.4	62	260	30	3.5
Wheat flour (85%)	346	13	11	1.6	72	213	20	2.5
" " (70%)	351	13	10	1	72.5	73	16	2
Wheaten bread,								
" White "	260	32	7.9	0.7	54	73	23	1.0
" Brown "	229	37	8.4	1.6	44	213	30	2.7
Biscuits								
" Cream cracker "	579	3.5	9.3	33	57.5	82	18	0.96
" Rusk "	408	6.4	6.6	8	74	81	87	2.66
" Water "	462	0.6	12	12.5	73	87	22	0.94
<i>Miscellaneous</i>								
Chocolate, plain.	562	tr.	4.6	32.5	52	139	26	3.28
Cocoa, powder	464	2.5	20.4	25.6	35	685	51	14.3
Coffee, ground	311	4	12.5	15.4	28.5	161	133	4.1
Tea, Indian	58	9.3	14	0	0	628	426	15.2
" Carrageen moss "	30	14	6.8	tr.	0.4	205	845	8.88
Honey	315	23	0.6	tr.	76.4	17	5	0.99
Marmalade	285	28	0.1	0	69.5	12	35	0.58
Sugar, white	409	0	0	0	99.9	tr.	1	0.04
Treacle, black	281	28.5	1.2	0	67	31	495	9.17
Margarine	794	13.7	0.2	85.3	0	12	4	0.3
Olive oil	929	tr.	tr.	99.9	0	tr.	tr.	0.08
Mushroom, raw	7	91.5	1.8	tr.	0	136	3	1.0
Bran	169	10	16	6	12	1,215	120	8.52
<i>Fruit, fresh :</i>								
Apple	50	84	0.2	tr.	12	8	3	0.2
Apricot	30	87	0.6	"	6.7	21	17	0.37
Banana	83	71	1.1	"	19	28	6	0.4
Blackberry	32	82	1.3	"	6.4	24	63	0.85
Cherry	51	71	0.6	"	12	17	16	0.4
Currant, black	31	77	0.9	"	6.6	43	60	1.27
Gooseberry	18	90	1.1	"	3.4	34	28	0.32
Grape	66	81	0.6	"	15.5	16	4	0.34
Grapefruit	24	91	0.6	"	5.3	16	17	0.26
Lemon	16	85	0.8	"	3.2	21	107	0.35
Melon, yellow	23	94	0.6	"	5	8	14	0.24
Orange	38	86	0.8	"	8.5	24	41	0.33
Pear	46	83	0.2	"	10.8	10	8	0.19
Plum, Victoria	42	84	0.6	"	9.6	16	11	0.36
Pineapple	50	84	0.5	"	11.6	8	12	0.42
Raspberry	27	83	0.9	"	5.6	29	41	1.21
Strawberry	28	89	0.6	"	6.2	23	22	0.71
Tomato (raw)	23	93	1	0.3	4	21	9	0.4
<i>Fruit, dried :</i>								
Apricot	198	15	4.8	tr.	43.4	118	92	4.1
Currant	266	22	1.7	"	63	40	95	1.82
Date	270	15	2	"	64	64	70	1.6
Fig	232	17	3.6	"	53	92	28	4.17

<i>Foodstuff, 100 gm. (3½ oz.).</i>	<i>Energy, in kilocal.</i>	<i>Water, in gm.</i>	<i>Protein, in gm.</i>	<i>Fat and other lipides, in gm.</i>	<i>Carbohydrate, available, in gm.</i>	<i>Phosphate, as P, in mg.</i>	<i>Calcium, in mg.</i>	<i>Iron, in mg.</i>
Olive (preserved) . . .	106	76	0.9	11	0	17	61	1.0
Prune . . .	175	23	2.4	tr.	40	83	38	2.9
Raisin . . .	269	21	1.1	„	64.4	33	61	1.55
<i>Nuts :</i>								
Almond, sweet . . .	600	5	20.5	53.5	4.3	442	247	4.23
Barcelona nut . . .	388	7	8	38	3	299	70	1.5
Brazil nut . . .	645	9	13.8	61.5	4.1	592	176	2.8
Chestnut . . .	185	52	2.3	2.7	36.6	74	46	0.89
Cob . . .	400	41	9	36	6.8	229	44	1.06
Coconut . . .	366	42	3.8	36	3.7	94	13	2.08
Peanut . . .	606	4	28	49	8.6	365	61	2.04
Walnut . . .	551	23	12.5	51.5	5	510	61	2.35
<i>Green Vegetables :</i>								
Asparagus (boiled) . .	18	92	1.1	tr.	3.4	84.5	36	0.9
Brussel sprout (boiled) .	17	91	2.4	„	1.7	45	27	0.6
Cabbage (boiled) . . .	9	96	0.8	„	1.3	24	45	0.46
Cauliflower (boiled) . .	11	95	1.5	„	1.2	33	23	0.48
Celery (raw) . . .	9	93	0.9	„	1.3	32	52	0.6
Leek (boiled) . . .	26	91	1.8	„	4.6	27.5	60	2.0
Lettuce (raw) . . .	12	95	1.1	„	1.8	30	26	0.2
Marrow (boiled) . . .	7	98	0.4	„	1.4	13	13	0.7
Spinach (boiled) . . .	27	85	5.1	„	1.4	93	560	4.0
Watercress (raw) . . .	14	91	0.7	„	0.7	52	222	1.6
<i>Roots and tubers (boiled) :</i>								
Artichoke, Jerusalem . .	20	36	1.6	tr.	3.2	33	30	0.41
Beetroot . . .	48	83	1.8	„	9.9	35.6	30	0.7
Carrot . . .	20	87	0.6	„	4.3	17	37	0.37
Onion . . .	14	97	0.6	„	2.7	16.4	24	0.25
Parsnip . . .	62	83	1.7	„	13.5	32	35	0.45
Potato . . .	87	80	1.4	„	19.7	29	4	0.48
Swede . . .	19	92	0.9	„	3.8	18	41	0.29
Turnip . . .	12	94	0.7	„	2.3	19	55	0.35
<i>Pulses (boiled) :</i>								
Bean, broad . . .	46	84	4.1	tr.	7.1	99	21	0.98
„ butter . . .	283	70	19.2	„	50	86.5	19	1.67
„ French . . .	8	95	0.8	„	1.1	15	39	0.59
„ haricot . . .	95	70	6.6	„	16.6	122	64	2.5
Lentil . . .	103	72	6.8	„	18.3	80	10	2.2
Pea, green . . .	52	80	5.0	„	7.7	83	13	1.2
„ dried . . .	107	70	6.9	„	19	113	24	1.4
Soy bean (dry) . . .	417	12	36	18	26	580	206	0.78

The tabulated values are selected from data compiled by Mottram and Radloff (1937), McCance and Widdowson (1940), Wokes (1941), and the Council of British Societies for Relief Abroad (1945).

CHARACTERISTICS OF THE CHIEF FOOD TYPES

1. **Animal Products.**—**Meat**, which is chiefly skeletal muscle, is characterised by : (a) a high protein value, usually of the order of 20 per cent., which may be concentrated by cooking ; (b) a variable fat value, up to about 30 per cent. ; (c) a negligible carbohydrate value ; (d) an iron value of about 2 mg. per 100 gm., much of which is in the almost unavailable forms of myohæmatin and hæmoglobin ; (e) a significant amount of the vitamins B₁, B₂, and nicotinic amide ; (f) inorganic and organic "extractives," one of which, glutamic acid, is partly responsible for the taste of roast meat. **Myosin**, the principal protein in meat, is the chief protein in the ordinary mixed diet, which includes about 15 to 20 gm., *per diem*. It contains all the known indispensable amino acids. **Glandular organs**, especially liver and kidney, are rich in nucleoprotein, and are valuable sources of the vitamins of the B group and the micro-essential metals Fe and Cu.

Fish resembles mammalian tissue in general composition, but is usually much poorer in fats, with the notable exception of species such as herring, mackerel, salmon and eel, which have a fat value of 10 to 15 per cent.

2. **Milk and Milk Products.**—Cow's milk provides high-grade protein (3 per cent.), fat rich in unsaturated fatty acids (3·6 per cent.), phosphate and calcium, and is an important but variable source of vitamins A and B.

Representative values, per 100 gm. fresh English pasture milk, are : A and carotenes, 80–250 i. units ; B₁, 0·05 mg. ; B₂, 0·15 mg. ; nicotinic acid, 0·08–0·4 mg. ; C, 1–3 mg. ; pantothenic acid, 0·4 mg. ; B₆, 0·03 mg. ; inositol, 14 mg. ; D, 2 i. units.

Good milk has 3·6 per cent. of fats, the legal minimum being 3·0 per cent.

Condensed milk has a protein value of 7 to 10½, and a sugar value that may be raised to 60 per cent. by addition of sucrose. Spray-dried milk powder has a protein value of about 26 per cent., and a water-content of 2–4 per cent.

The *specific gravity* of cow's milk when fresh is 1·029–1·033 ; the *freezing-point*, as estimated by the cryoscope, is almost constant within the limits — 0·53° C. and — 0·57° C. These properties are important in deciding the quality of a sample of milk.

Butter has 85–90 per cent. of fat, which carries the fat-soluble vitamins present in the milk, so that 1 oz. of butter is equivalent to the vitamin A and D values of about 1½ pints of the original milk.

Bacharach (1940) suggests that a reasonable standard for summer butter is not less than 50 i. units of A and provitamin A per gram

(1,400 units per oz.), and not less than 0.75 i. units of D per gram (21 units per oz.). The vitamin D value of butter is seldom more than 1 unit per gram, and may be as low as 0.01 unit, in winter butter.

Cheese is a concentrated foodstuff, with a protein value of 20-40 per cent., and a fat value of 15-40 per cent. It is an important source of phosphate and calcium (0.3-1.2 per cent.), and vitamin A (10-50 units per gram).

3. Vegetables.—With the exception of the tubers and dried pulses, the vegetables are poor sources of energy, but are important protective foodstuffs, capable of supplying all the dietary requirements for vitamin C, most of the requirements for provitamin A, and providing some iron and calcium, as well as magnesium, which is released from the otherwise unassimilated chlorophyll. Much of the carbohydrate present is in the unavailable form of pentosans and cellulose.

Potato is a very important foodstuff on account of its low cost, its availability and popularity. In addition to its high starch content, which is about 20 per cent., potatoes contain 0.7-3.6 per cent. of protein, half of which is the globulin *tuberin*, of high nutritional value.

Vitamin values, per 100 gm. fresh tuber, are: B₁, 0.02-0.3 mg.; B₂, 0.007-0.06 mg.; C, 5-40 mg. Although the C value is relatively low and variable, it is significant because of the amount of material eaten, and 1 lb. of potatoes can supply 20 to 150 mg. of ascorbic acid. The fate of the vitamin depends on the method of cooking. The loss on steaming is slight; slicing and long period boiling may reduce the value to zero.

The composition of the chief individual foodstuffs is surveyed in a series of important reviews in *Chemistry and Industry*, Vol. 59 (1940) to Vol. 61 (1942).

APPENDIX II

REAGENTS EMPLOYED IN BIOCHEMISTRY

(In aqueous solution, unless otherwise stated.)

Antimony trichloride, 30 per cent. in chloroform. Reagent for vitamin A and other carotinoids (p. 224).

Benedict's Qualitative Copper Reagent: 17.3 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, with 173 gm. Na citrate and 100 gm. anhydrous Na_2CO_3 in 1,000 ml. water. Used for detecting reducing sugars.

Benedict's Quantitative Copper Reagent: 18.0 gm. crystalline $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, with 200 gm. Na citrate, 125 gm. KSCN, 5 ml. of 5 per cent. K ferrocyanide, and 100 gm. anhydrous Na_2CO_3 . Dissolve separately, mix, and make up to 1,000 ml. with water. Before use, add 3-5 gm. anhydrous Na_2CO_3 to 25 ml. of reagent. Used for estimating reducing sugars.

Benzidine, fresh saturated solution in glacial acetic acid. Reagent for blood pigment in urine, and for nitrate in saliva.

Diazo-Reagent (Ehrlich, Van den Bergh), freshly prepared mixture of 1 ml. of 0.5 per cent. NaNO_2 and 50 ml. of 0.1 per cent. sulphanilic acid in 2 per cent. HCl. Reagent (in acid solution) for free bilirubin; and (in alkaline solution) for histidine, histamine, tyrosine, tyramine, indoxyl, and polyphenols.

2:6-Dichloroquinone-chloroimide, 0.4 per cent. in absolute alcohol. Kept in brown glass bottles and in the dark, the reagent is stable for at least three months. In alkaline solution (pH 9-10 is best) it gives colours with uric acid (yellow), phenols unsubstituted in the *para*-position (violet-blue), indole, methylamine, glycine, thiourea (violet).

3:5-Dinitrobenzoic Acid, 5 per cent. Yields a purple colour with creatinine in alkaline solution. The reagent may be used in alcoholic solution, or dissolved in dilute sodium carbonate.

Ehrlich's Aldehyde Reagent, 2-3 per cent. *p*-dimethylamino benzaldehyde in alcohol or in 20 per cent. HCl. Reagent for mucoproteins, indole, indoxyl, urobilinogen, urea, allantoin; and (in excess of strong acid) tryptophane and scatole. The reagent in 20 per cent. HCl is almost colourless, and is suitable for the urea and allantoin tests.

Fehling's Reagent. See p. 125.

Folin's Reagent, for uric acid and phenols. Boil under a reflux condenser for two hours a mixture of: 100 gm. sodium tungstate,

102 ml. *ortho*-phosphoric acid (B.P. 66.3 per cent.), and 750 ml. of water. When the mixture has cooled, sufficient bromine is added to bleach completely any blue colour. The mixture is then boiled to remove the excess of bromine, cooled and made up to 1 litre with water.

Guaiacum, 2 per cent. in alcohol. With H_2O_2 , it is a reagent for peroxidases, and hæmoglobin in urine.

Glyoxylic Acid Reagent. Add slowly 4 gm. of powdered Mg. to 100 ml. saturated oxalic acid. Filter when reduction is complete, and dilute with water to 400 ml. Used as a ring test with H_2SO_4 for the detection of tryptophane in proteins. Also gives colours with indole and scatole.

H-ion Indicators. These reagents change colour sharply at a particular concentration of H-ions. A large number are in practical use, covering the acid-alkali scale from pH 0.1 to about pH 13. For details of application consult: *Practical Physiological Chemistry*, by S. W. Cole; *H-ion Concentration*, by F. L. LaMotte, W. R. Kenny, and A. B. Reed; *Quantitative Inorganic Analysis*, by A. I. Vogel.

H-ion Indicators

Indicator.	pH Range ←Acidity—Alkalinity→	
Methyl violet . . .	yellow	0.1—1.5 green
Thymol blue. . . .	red	1.4—2.8 yellow
Methyl violet	green	1.5—3.2 violet
Tropæolin OO	pink	1.5—3.0 yellow
Methyl yellow	red	2.9—4.0 yellow
Methyl orange	orange	3.0—4.4 yellow
Tetrabromphenol blue	yellow	3.0—4.6 blue
Bromcresol green	yellow	3.8—5.4 blue
Methyl red	red	4.3—6.2 yellow
Litmus	red	5.0—8.0 blue
Tashiro's indicator	pink	5.45—5.50 green
Bromthymol blue	yellow	6.0—7.6 blue
Phenol red	yellow	6.7—8.3 red
Thymol blue	yellow	8.0—9.6 blue
Phenolphthalein	colourless	8.2—10.0 red

These indicators are generally used in 0.04 per cent. aqueous solution, with the exception of phenolphthalein, which is dissolved in alcohol.

For gastric analysis and titration stronger solutions (1 per cent.) of methyl violet, thymol blue, and methyl yellow are used.

Methyl yellow is also known as Töpfer's indicator or dimethyl-amino-

azo-benzene. It will be seen that some of the indicators have an extended pH range in that they show more than two colours as the reaction changes from acidity to alkalinity. Examples of these indicators are methyl violet, which changes from yellow, green, blue, to violet, and thymol blue, which changes from red, yellow, green, to blue.

This property is developed in the use of mixed reagents, such as the B.D.H. "universal indicator," which has the following colour range:

pH up to 3.0	Red	pH 8.0	Green
" 4.0	Deeper Red	" 8.5	Bluish Green
" 5.0	Orange Red	" 9.0	Greenish Blue
" 5.5	Orange	" 9.5	Blue
" 6.0	Orange Yellow	" 10.0	Violet
" 6.5	Yellow	" 10.5	Reddish Violet
" 7.0—7.5	Greenish Yellow	" 11.0	Deeper Reddish Violet

Litmus is included in the list to show how insensitive it is, relatively, when compared with the newer reagents. A change of three pH degrees is required to alter it from red to blue. Phenol red is a much sharper indicator for titrations to the end-point of absolute neutrality, pH 7.0.

For general use, one drop of indicator is added to each millimetre of solution examined. In exact work, the resulting colour is matched with a standard in a comparator.

Tashiro's Indicator. The stock solution is made by mixing 200 ml. of 1 per cent. alcoholic methyl red with 50 ml. of 0.1 per cent. alcoholic methylene blue. Both dyes must be dissolved in pure alcohol. For use, 1 part of stock solution is mixed with 1 part of alcohol and 2 parts of distilled water. This is an example of a *screened* indicator, or one in which the colour change is made more distinct by addition of a second pigment (methylene blue) to cut out part of the spectrum. The indicator is used in the Conway methods for the micro-analysis of ammonia and urea.

Fluorescent Indicators. These reagents respond to a change in pH by changing in colour or in intensity of fluorescence when exposed to ultra-violet light, and are very useful for analysis involving darkly-coloured liquids. The solution to be titrated is placed in a thin-walled flask or beaker on a black surface screened from sunlight. During the titration the mixture is illuminated obliquely by a beam of ultra-violet light to show the change in fluorescence.¹

¹ An ultra-violet lamp suitable for use while titrating has been introduced by Messrs. Baird and Tatlock (London) Ltd., and it and the appropriate fluorescent indicators can be obtained from Messrs. Hopkin and Williams Ltd., of 16 St. Cross Street, London.

Fluorescent Indicators

Indicator.	pH Range ←Acidity—Alkalinity→	
3 : 6-Dihydroxy-phthalimide	colourless	0.0—2.5 yellow-green
	yellow-green	7.5—8.5 green
3 : 6-Dihydroxy-	colourless	0.2—1.5 blue
phthalonitrile	blue	6.0—8.0 green
Fluorescein	green	0.1—0.5 yellow
Eosin	red	2.0—3.5 yellow
Erythrosin B	red	3.0—5.0 yellow
Acridine	green	4.5—5.5 blue
Quinine sulphate	blue	4.5—8.5 violet
	violet	8.5—9.5 colourless
β -Naphthol	colourless	6.0—6.5 blue

Methylene Blue, 1 per cent. Redox indicator used in determining the end-point in Fehling's sugar estimation.

Millon's Mercury Reagent. 3 gm. Hg dissolved in 4 ml. concentrated HNO_3 , diluted to 10 ml. with water, and filtered. Used for detecting tyrosine in proteins and phenols in urine.

α -Naphthol, 2 per cent. in alcohol. General reagent for carbohydrates, also used to detect arginine in proteins and indoxyl in urine.

β -Naphthoquinone Sulphonate, 10 per cent. General reagent for proteins and amino acids.

Naphtho-resorcinol, 1 per cent. in alcohol. Special reagent for glycuronic acid in urine.

Ninhydrin, 0.2 per cent. General reagent for proteins and amino acids.

Phenylhydrazine Hydrochloride: Osazone reagent for sugars.

Potassium Chromate, 5 per cent. With concentrated HNO_3 , reagent for all primary and secondary alcohols, including sugars, glycerol, lactic acid, β -hydroxy-butyric acid, and tartaric acid.

Resorcinol, 5 per cent. in alcohol. Reagent for ketoses and indoxyl.

Schiff's Rosaniline Reagent, 1 per cent. rosaniline decolourised by SO_2 . General reagent for free aldehyde groups.

Salicylsulphonic Acid, 20 per cent. General precipitant for higher proteins.

Sodium Nitroprusside (Nitrosoferricyanide), 5 per cent. Test for sulphhydryl compounds, including cysteine, thioneine, and glutathione. Also reacts with acetone, acetoacetic acid, creatinine and indole.

The reagent is unstable if exposed to light, and should be kept in the dark or stabilised by addition of a couple of drops of nitric acid.

Standard Sugar Solution (Lane and Eynon). Dissolve exactly 9.5 gm. of pure sucrose in 100 ml. of approximately 2 per cent. (or 0.6 Normal) HCl. The mixture is kept for a week, during which time it undergoes hydrolysis to "invert" sugar, an equimolecular mixture of glucose and fructose. It is then diluted to 1 litre and stored. For standardisation, 50 ml. is neutralised and made up to 100 ml. with water to yield a 0.5 per cent. solution of "invert" sugar.

Thymol 3 per cent. in alcohol. General reagent for carbohydrates. Also reacts with indoxyl.

Xanthidrol, 10 per cent. in methyl alcohol. Reagent for urea and indole.

ABBREVIATIONS AND EQUIVALENTS

Weight

- 1 kilogram, kg. = 2.2046 lb. = 35.274 oz. avoirdupois.
 1 gram, gm., or g. = 0.001 kg. = 15.432 gr. = 0.03527 oz. avoirdupois.
 1 milligram, mg. = 0.001 gm. = 10^{-6} kg.
 1 microgram, μ g., or γ = 0.001 mg. = 10^{-6} gm.
 1 pound avoirdupois, lb. = 7,000 grains = 16 oz. avoirdupois
 = 453.59 gm.
 1 ounce avoirdupois, oz. = 16 drams = 437.5 gr. = 28.35 gm.
 1 grain, gr. = 64.8 mg.
 1 ton = 2,240 lb. = 1,006 kg.
 1 metric ton, or tonne = 1,000 kg. = 2,204 lb. = 0.9842 tons.

Length

- 1 metre, m. = 1.0936 yards = 3.281 ft. = 39.37 in.
 1 centimetre, cm. = 0.01 m. = 0.394 in.
 1 millimetre, mm. = 0.001 m. = 0.0394 in.
 1 micrometer, or micron, μ = 0.001 mm. = 10^{-6} m.
 1 millimicron, or micromillimetre, $m\mu$ = 0.001 μ = 10^{-6} mm.
 1 Ångström unit, Å. = 0.1 $m\mu$.
 1 kilometer, km. = 1,000 m. = 0.6214 miles = 3,280.83 ft.
 1 yard, yd. = 3 ft. = 0.9144 m.
 1 foot, ft. = 12 in. = 30.48 cm.
 1 inch, in. = 2.54 cm.
 1 mile = 5,280 ft. = 1.6093 km.

Volume

- 1 litre, l. = volume at 4° C. of 1,000 gm. of water
 = 1,000.027 cubic cm. (c.c.).
 = 0.22 gallons (British) = 0.2642 gallons (U.S.A.).
 = 1.76 pints (British) = 2.11 pints (U.S.A.).
 = 35.196 fluid oz. (British) = 33.8 fluid oz. (U.S.A.).
 1 millilitre, ml. = 0.001 l., or, approximately, 1 c.c.
 = 0.0352 fluid oz. (British) = 16.9 minims.
 1 microlitre, μ l. = 0.001 ml. = 1 cubic mm. = 10^{-6} l.
 1 cubic metre, cu.m. = 1,000 l.
 1 gallon (British), gal. = volume at 62° F. of 10 lb. of water.
 = 70,000 gr. = 4.54 l.
 = 1.201 gal. (U.S.A.) = 8 pints (British).
 1 gallon (U.S.A.) = 8.313 lb. of water = 8 pints (U.S.A.) = 3.785 l.
 1 pint (British) = 20 fluid oz. = 0.568 l.
 1 fluid oz. (British) = 28.41 ml. = 8 fluid drachms = 480 minims.
 1 minim = 59 μ l. = 0.0592 ml. = 0.96 minims (U.S.A.).

Miscellaneous

mol, or mole, M = formula-weight of a substance (ions or molecules) expressed in gm.; the weight in gm. numerically equal to the ionic or molecular weight.

millimol, mM. = 0.001 mol.

molar solution = 1 mol. per l. of solution.

molar concentration, $[]$ = the number of mols. present per l. of solution.

gram-equivalent, gm.-eq. = mol value divided by valency value; the ionic weight in gm. divided by the valency.

normal solution, N . = 1 gm. eq. per l. of solution.

normal acid = 1 mol available H^+ per l. = 1 gm. available H^+ per l.

normal alkali = 1 mol available OH^- per l. = 17 gm. available OH^- per l.

$[H^+]$, C_H = hydrogen ion concentration, in mol H^+ per l.

$[OH^-]$, C_{OH} = hydroxyl ion concentration, in mol OH^- per l.

pH = hydrogen ion exponent = $-\log_{10} [H^+]$; $[H^+] = 10^{-pH}$.

pK_a = acid-dissociation exponent = $-\log_{10} K_a$.

1 atmosphere, atm. = 14.7 lb. per sq. in. = 1,033 gm. per sq. cm.
= 33.9 ft. of water at 4° C. = 760 mm. Hg. at 0° C.

1 calorie, gram-calorie, or small calorie, gm. cal. = quantity of heat required to raise the temperature of 1 gm. of water through 1° C. (from 14.5° to 15.5° C., for *normal* gm. cal.).

1 kilogram-calorie, kilocalorie, or large calorie, kilocal., or kg. cal.
= 1,000 gm. cal., = 41.8×10^{10} ergs.

Freezing-point of pure water at 1 atm. = 0.0° C. = 32° F.

Temperature of maximum density of water = 4.0° C. = 39.2° F.

Boiling-point of pure water at 1 atm. = 100° C. = 212° F.

Temperature scale conversion: Centigrade to Fahrenheit; multiply ° C. by 9, divide by 5, and add 32.

Fahrenheit to Centigrade; subtract 32 from ° F., multiply by 5, and divide by 9.

Human body temperature, normal level, approx., 36.9° C., or 98.4° F.

Respiratory quotient, $R.Q.$ = CO_2/O_2 = ratio of the volume of liberated CO_2 to volume of O_2 absorbed during a given period.

Respiratory coefficient, tissue respiration quotient, Q_o - or Q_{CO_2} = volume in microlitres of gas absorbed or liberated per hour by an amount of tissue representing 1 mg. dry weight.

parts per million, p.p.m. = mg. per kg. = gm. per cubic m.

grains per gallon = 1 part per 70,000, for aqueous solutions.

Wavelength, λ = distance in $m\mu$ or in \AA between corresponding phases of two consecutive waves = speed divided by frequency (number of vibrations per second).

Electromagnetic radiation, vibration-effect transmitted at the speed of 3×10^{10} cm. per sec., the quality of which depends on the wavelength.

1. electrical waves, λ = 30 km. to 1 mm.
2. infra-red λ = 1 mm. to 760 $m\mu$.
3. visible λ = 760 $m\mu$ (red) to 400 $m\mu$ (violet).
4. ultra-violet λ = 4,000 \AA . to 5 \AA .
5. X-rays λ = 5 \AA to 0.1 \AA .
6. α -rays λ = 0.1 \AA to 0.01 \AA .
7. cosmic rays λ = 0.01 \AA to 0.000,01 \AA .

Extinction coefficient, $E = \log_{10} \frac{I_0}{I}$ where I_0 is the intensity of the incident light, and I is the intensity of the emergent light. Suffixes are added to denote: the thickness of the absorbing layer, the concentration of the solute, and the wavelength of the absorbed light.

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*How index-learning turns no student pale,
Yet holds the eel of science by the tail.*

(ALEXANDER POPE)

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